AOP ID and Title:

SNAPSHOT

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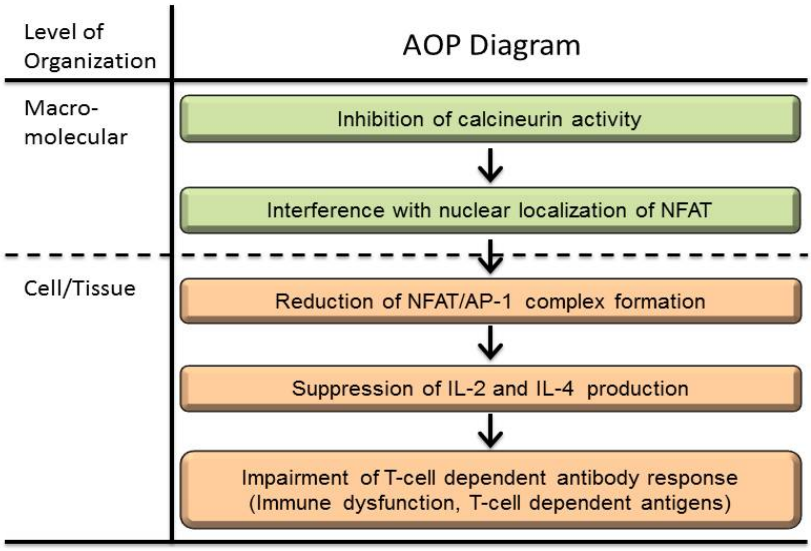
AOP 154: Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response

Short Title: Immunosuppression

Graphical Representation



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Status

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 Do not cite	EAGMST Under Review	1.38	Included in OECD Work Plan

Abstract

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

CN inhibitors (CNIs) inhibit CN phosphatase activity to suppress many kinds of immune functions. T-cell dependent antibody response (TDAR) is considered to be the most important endpoint on evaluating immunotoxicity of chemicals; therefore, this AOP describes the linkage between the inhibition of CN activity and impairment of TDAR.

CN activity is inhibited when stressors of CNIs bind to CN with their respective immunophilins, which interferes with the nuclear localization of nuclear factor of activated T cells (NFAT), a substrate of CN. As a result, the formation of functional NFAT complexes with activator protein-1 (AP-1) that bind at the site of IL-2, IL-4 and other T cell -derived cytokine promoters is reduced, thereby suppressing production of these cytokines. Thus, reduced production of IL-2 and IL-4 affects the proliferation and differentiation of B-cells to suppress TDAR.

We have identified a number of key events along this pathway and determined the key event relationships, based on which we have created an AOP for inhibition of CN activity leading to impaired TDAR.

Since CN expresses in cells among vast variety of species, this AOP might be applicable to many mammal species, including humans and rodents.

Background

Although there are numerous stressors that inhibit CN activity, this AOP is mainly 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 more clarify the linkage between inhibition of CN activity and impairment of TDAR.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	980	Inhibition, Calcineurin Activity (https://aopwiki.org/events/980)	Inhibition, Calcineurin Activity
2	KE	979	Interference, nuclear localization of NFAT (https://aopwiki.org/events/979)	Interference, nuclear localization of NFAT
3	KE	981	Reduction, NFAT/AP-1 complex formation (https://aopwiki.org/events/981)	Reduction, NFAT/AP-1 complex formation
4	KE	1202	Suppression, IL-2 and IL-4 production (https://aopwiki.org/events/1202)	Suppression, IL-2 and IL-4 production
5	AO	984	Impairment, T-cell dependent antibody response (https://aopwiki.org/events/984)	Impairment, T-cell dependent antibody response

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Inhibition, Calcineurin Activity (https://aopwiki.org/relationships/1508)	adjacent	Interference, nuclear localization of NFAT	High	High
Interference, nuclear localization of NFAT (https://aopwiki.org/relationships/1017)	adjacent	Reduction, NFAT/AP-1 complex formation	High	High
Reduction, NFAT/AP-1 complex formation (https://aopwiki.org/relationships/1509)	adjacent	Suppression, IL-2 and IL-4 production	High	High
Suppression, IL-2 and IL-4 production (https://aopwiki.org/relationships/1510)	adjacent	Impairment, T-cell dependent antibody response	High	High

Stressors

Name	Evidence
Tacrolimus	
Cyclosporin	

Overall Assessment of the AOP

CN phosphatase activity is inhibited when stressors bond to Calcineurin-A (CnA) with immunophilins, which interferes with the nuclear localization of NFAT, a substrate of CN. As a result, the formation of functional NFAT/ AP-1 complexes that bind at the site of IL-2 , IL-4 and other cytokine promoters is reduced, thereby suppressing production of these cytokines. Thus TDAR is impaired mainly by the suppression of production of IL-2 and IL-4, which affect the proliferation and differentiation of B-cells to lower TDAR. We have identified a number of key events (KEs) along this pathway, and based on these key event relationships (KERs), created an AOP for inhibition of CN activity leading to impaired TDAR.

Since each KE involving MIE and AO is quantifiable, and shows similar dose responses with the CNIs in vitro, this AOP is useful for understanding immunosuppression due to inhibition of CN activity. In addition, each KER is based on sufficient scientific evidence and exhibits no contradiction with dose responses of adjacent KEs.

Since CN/NFAT system expresses in cells among vast variety of species and the function in immune system is common in at least human and mice, this AOP might be applicable to many mammalian 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	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Mus musculus	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Macaca fascicularis	Macaca fascicularis	High	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	High

The proposed AOP regarding inhibition of CN activity leading to impaired TDAR is not dependent on life stage, sex, or age. Since tacrolimus (FK506) ointment (Protopic) is approved for pediatric atopic dermatitis, the MOA for immunosuppression appears to be applicable to all life stages. Since FK506 or Cyclosporine A (CsA)-induced outcomes in humans are mimicked by similar responses in a variety of animal models including non-human primates and rodents, immunosuppression induced by inhibition of CN activity is considered to occur across a variety of mammalian species.

Essentiality of the Key Events

MIE and later events: CnA-knockout (KO) mice

The CN molecule consists of two regions, CnA and CnB, of which CnA exhibits phosphatase activity. In CnA-KO mice, T-cell proliferation in response to ovalbumin stimulation is lower than that for wild-type mice and is not complemented by normal antibody producing cells. In addition, when stimulated with ovalbumin, CnA-KO mice produce less IFN-γ, IL-2, and IL-4 than wild-type mice. However, primary antibody response in CnA-KO mice is normal in response to TNP-ovalbumin, which means that CnA deficiency affects only on T cell-dependent antibody response (TDAR).

Stressor: FKBP12-KO mice

FK506 induces suppression of immune responses; however, there is no evidence of a relationship between FKBP12 knockout and the immune system in the FKBP12-KO mouse model. Steric structure of FKBP12/FK506 complex is the key factor for inhibition of CN phosphatase activity, but not for the enzymatic activities of FKBP12.

KE1 and later events: NFAT-KO mice

The following phenotypes are observed in NFAT-KO mice: moderate hyperproliferation with splenomegaly, moderately enhanced B- and T-cell responses, with bias towards Th2-cell response, decreased IFN-γ production in response to T-cell receptor (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, profound defects in cytokine production and cytolytic activity, B-cell hyperactivity, impaired development of CD4 and CD8 single-positive cells, increased apoptosis of double-positive thymocytes, and mild hyperactivation of peripheral T cells.

Therefore, the study of NFAT-KO mice shows that NFAT is involved in a wide range of immune responses, and some of these phenomenon are known to be regulated by CN. Suppression of T-cell-derived cytokines is noted both in CnA-knockout and NFAT-knockout mice, which indicates that the production of T-cell derived cytokines such as IL-2 and IL-4 is regulated by the CN-NFAT system.

Weight of Evidence Summary

Biological Plausibility

T-cell functions are mainly regulated by the CN-NFAT system and suppression of CN activity in T cells is known to induce multiple types of immunosuppression, including T cell-dependent antibody response (TDAR).

Experiments with T cells indicate that TCR stimulation brings about increases in intracellular concentrations of Ca2+ that trigger CN activity, thereby inducing nuclear localization of substrate NFAT per dephosphorylation. The localized NFAT forms complexes with activator protein 1 (AP-1) at the promoter sites of the T-cell cytokine genes and induces production of the cytokines.

CN phosphatase activity is known to be inhibited by the formation of immunophilin-CN inhibitor (CNI) complexes, such as CsA/cyclophilin complexes or FK506/FK506-binding protein (FKBP) 12 complexes. Immunophilins are a general class of proteins that exhibit peptidyl-propyl isomerase (PPIase) activity, but there is no commonality between amino-acid sequences of the two classes of immunophilins. The three-dimensional structures of immunophilin complexes are essential to the inhibition of CN phosphatase activity, even though their enzymatic activities are not.

It is also known that one of the effects on immune function when CNI forms complexes with its respective immunophilin and inhibits CN activity is the suppression of IL-2 and other T-cell derived cytokine production. It is further known that inhibition of CN leads to suppression of TDAR because IL-2 and IL-4 mainly promote the proliferation, class switching, differentiation, and maturation of B-cells.

Furthermore, CN-NFAT also exists in B-cells and it has been reported that CNIs do suppress production of certain cytokines from them. At the time of our review of the literature, however, we did not find any reports of a direct effect of CN inhibition on B-cells, such as changes in proliferation, class switching, differentiation, or maturation of B-cells.

Also, although CN-NFAT is known to exist in dendritic cells, natural killer T (NKT) cells, and other types of cells in which it regulates the expression of IL-2 receptors, there are no reports of effects on the production of T cell-dependent antibodies due to CNI-induced alteration in expression of IL-2 receptors in these cells.

CN-NFAT system-mediated immunosuppression is well understood based on the pharmacology of some CNI drugs; therefore, AOP of CN inhibition-induced suppression of TDAR is useful for prediction of CN-mediated immunotoxicity.

KER	KE_{up}-KE_{down}	Plausibility	Rationales supported by literatures
KER1	CN inhibition to interference, NFAT nuclear translocation	Strong	T cell functions are regulated by CN-NFAT system. CN phosphatase activation through TCR stimulation dephosphorylates NFAT, thereby promoting nuclear localization of NFAT. CN phosphatase activity in T cells could be inhibited by CNI/immunophilin complexes, thus interfering with dephosphorylation and nuclear localization of NFAT.
KER2	Interference, nuclear localization to reduction, NFAT/AP-1 complex formation	Strong	CN activity dephosphorylates NFAT, thereby promoting its nuclear translocation. Nuclear-located NFAT binds with AP-1 at the promoter regions of the cytokine genes to promote T-cell cytokine production. Inhibition of dephosphorylation of NFAT by CNIs prevents nuclear localization of NFAT and resultant binding with AP-1 at the promoter region of the T cell cytokine genes.

KER3	Reduction, NFAT/AP-1 complex formation to suppression of IL-2 and IL-4 production	Strong	NFAT/AP-1 complexes bind to the promoter regions of the cytokine genes, which promotes production of cytokines from T cells. Of these cytokines, IL-2 and IL-4 have a major role in promoting proliferation, maturation and class-switching of B cells, and development of TDAR. Reduction of NFAT/AP-1 complex formation in the nucleus due to inhibition CN activity by CNIs suppresses production of T-cell derived cytokines, including IL-2 and IL-4.
KER4	Suppression of IL-2 and IL-4 production to impaired TDAR	Strong	T cell-derived cytokines play important roles in TDAR. Among them, IL-2 promotes proliferation of B cells, and IL-4 affects maturation and class switching of B cells as well as proliferation. Inhibition of CN activity by CNIs is known to suppress production of multiple cytokine species from T cells. Of these cytokines and receptors, suppression of IL-2 and IL-4 production mainly leads to impairment of TDAR. Suppressed production of other cytokines due to inhibition of CN activity exhibits only minor effects, if any, on TDAR.

Empirical Support

KER	Empirical support of KERs
MIE=>KE1 Inhibition, calcineurin activity leads to interference, nuclear localization of NFAT	Empirical support of the MIE => KE1 is strong. Rationale MIE: CN phosphatase activity is inhibited by CNI of FK506 with IC50 values of 0.5 nM (FK506) and 5nM (CsA) after 1 hours treatment (Fruman et al.1992). KE1: Concentration-dependent reduction of in vitro nuclear localization of NFAT was evident at the concentration from 0.1 nM (Jurkat T cells) or 10nM (human CD4+ T cells) and up to 1 µM (1000 nM) under the conditions of 2 hours treatment (Maguire et al. 2013). CN phosphatase activity and nuclear translocation of NFAT seems to be suppressed by CNIs at the similar ranges of doses and reaction times of 1 to 2 hours.
KE1=>KE2 Interference, nuclear localization of NFAT leads to reduction, NFAT/AP-1 complex formation	Empirical support of the KE1 => KE2 is strong. Rationale KE1: Concentration-dependent reduction of in vitro nuclear localization of NFAT was evident at the concentration from 0.1 nM (Jurkat T cells) or 10nM (human CD4+ T cells) and up to 1 µM (1000 nM) under the conditions of 2 hours treatment (Maguire et al. 2013). KE2: Treatment of activated T cells with FK506 at 100ng/mL (125nM) or CsA at 500ng/mL (416nM) for 2 hours hinders the formation of functional NFAT/AP-1 in the nucleus (Flanagan et al. 1991). Quantitative data on NFAT/AP-1 complex formation in the nucleus is insufficient; however, inhibition of nuclear localization of NFAT and following NFAT/AP-1 complex formation in the nucleus are simultaneously detected by gel mobility shift assay at the concentration of FK506 within the range for inhibition of nuclear translocation of NFAT using imaging flowcytometry after 2 hours culture of T cells.

KE2=>KE3 Reduction, NFAT/AP-1 complex formation leads to suppression, IL-2 and IL-4 production	Empirical support of the KE2 => KE3 is moderate. Rationale KE2: Gel mobility shift assay revealed that treatment of activated T cells with FK506 at 100ng/mL (125nM) or CsA at 500ng/mL (416nM) for 2 hours hinders NFAT nuclear translocation and following formation of NFAT/AP-1 complexes in the nucleus (Flanagan et al. 1991). KE3: In CD3/PMA-activated human T cells, FK506 suppressed production of IL-2, IL-4, and IFN-γ at the concentrations of 1.2 to 12.5 nM after 22 to 24 hours culture as well as inhibited expression of IL-2, IL-4, and IFN-γ mRNA in a dose-dependent (10 nM) manner (Dumont et al. 1998). Therefore, concentration of CNI needed for inhibition of NFAT/AP-1 complex formation in the nucleus is higher than that for inhibition of IL-2 and IL-4 production.Time lag is found between the two KEs; 2 hours for KE2 and 22 to 48 hours for KE3.
KE3=>AO: Suppression, IL-2 and IL-4 production leads to Impairment, T-cell dependent antibody response	Empirical support of the KE3 => AO is strong. Rationale KE3: In CD3/PMA-activated human T cells, FK506-suppressed production of IL-2, IL-4, and IFN-γ at concentrations of 1.2 to 12.5 nM after 22 to 24 hours cultures as well as inhibited expression of IL-2, IL-4, and IFN-γ mRNA in a dose-dependent (10 nM) manner. (Dumont et al. 1998). KE4: After a 9-day culture of B cells and non-pre-activated T cell stimulation with FK506 or CsA, the levels of IgM and IgG in the culture supernatant were reduced at 0.3 and 1.0 ng/mL of FK506 or 50 and 100 ng/mL of CsA (Heidt et al, 2009).. After a 4-day culture of SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) and anti-CD3/CD28 stimulated PBMC culture supernatant with FK506 or CsA, the level of IgM in the culture supernatant was reduced at concentrations of 0.01 to 100 ng/mL of FK506 or 0.1 to 1000 ng/mL of CsA (Sakuma et al. 2001b). Rats were treated with FK506 for over four weeks and immunized with KLH, after which serum concentration of anti-KLH IgM and IgG was reduced at the dose level of 3 mg/kg/day (Ulrich et al. 2004). Mice were treated with FK506 or CsA for 4 days, and immunized with SRBC, after which antigen-specific plaque-forming splenocytes were reduced at dose levels of 3.2, 10, 32 and 100 mg/kg of FK506 or 32 and 100 mg/kg of CsA (Kino et al. 1987b). In vitro class switching; in CD3/PMA-activated human T cells, FK506 suppressed production of IL-2, IL-4, and IFN at the concentrations of 1.2 to 12.5 nM as well as inhibited expression of IL-2, IL-4, and IFN-γ mRNA at the concentrations of 10 nM (Dumont et al. 1998). In vitro suppression of T-cell–derived cytokines and T-cell–dependent antibody production or antibody production after polyclonal T-cell stimulation showed similar dose responses to CNIs. Time gaps were found, however, between these two events, which showed earlier onset of cytokine production and delayed onset of antibody production.

Based on these findings of empirical support, each KE involving MIE and AO except for KE2 shows similar dose responses to the CNIs in vitro; however, culture time lag is noted, in that, 1 hour for MIE, 2 hours for KE1 and KE2, 22 to 24 hours for KE3 and more than days for AO.

Quantitative Consideration

KER1

There have been no literature available to show clear quantitative relationship between the inhibition of CN phosphatase activity and nuclear translocation of NFAT; however, the dose responses of CN phosphatase activity and nuclear translocation of NFAT to CNI deem to be the same.

KER2:

Gel mobility shift assay of activated T cells showed that NFAT/AP-1 complexes are only found in nuclear extract, which indicates a strong relationship between the nuclear translocation of NFAT and simultaneous complex formation with AP-1 in the nucleus. CNI treatment clearly suppresses the complex formation of nuclear located NFAT and AP-1 in the nucleus, which also shows the solid relationship between these adjacent two KEs although quantitative data on suppressed NFAT/AP-1 complex formationis insufficient (Flaganan W.M. et al. 1991).

KER3:

The quantitative relationship between the decreased formation of NFAT/AP-1 complexes and the production of IL2/IL-4 formation induced by CNIs has not been reported.

However, as mentioned in the empirical support, nuclear localization of NFAT is strongly related to NFAT/AP-1 complex formation in the nucleus, and the dose responses of IL2/IL-4 production and nuclear translocation of NFAT inhibited by CNI are similar; therefore, dose ranges of CNI in the inhibitions of IL2/IL-4 production and NFAT/AP-1 complex formation in the nucleus might also be the same.

In addition, T-5224 and ursolic acid inhibit AP-1 DNA binding activity or production of NF-κB, NFAT and AP-1, respectively, and both suppress the IL-2 and/or IL-4 production with dose dependent manner including the doses of inhibiting NFAT-AP-1 system (Yoshida et al. 2015, Checker et al. 2012).

KER4:

Inhibition of IL-4 production in mice treated with oral administration of suplatast tosilate suppresses antigen-specific IgE production with a dose-dependent manner (Taiho Pharmaceutical 2013). In the inhibition of IL-4 production in human cell culture by suplatast tosilate at the concentration of 10 μg/mL for 10 days, antigen specific IgE production was suppressed from 56 to 72% and IL-4 production was suppressed from 58 to 76% (Taiho Pharmaceutical 2013).

As for IL-2 and antibody production, in vitro T-cell-induced polyclonal B cell activation to produce antibody was inhibited with anti-IL-2 and anti-IL-2R antibodies. T (Owens T, 1991). In addition, cynomolgus monkeys treated wth CsA showed suppression of IL-2 and TDAR using sheep red blood cells with a dose dependent manner (Gaidal K. 2015).

In the human T-B cell co-culture stimulated with anti-CD3 monoclonal antibody, CNIs of FK506 and CsA lowered the levels of T-cell cytokines including IL-2 and IL-4 and inhibited IgM and IgG productions with a dose-dependent manner (Heidt S. 2010).

These results show the quantitative relationships between the inhibition of IL-4 or IL-2 by specific antibodies or CNI and suppression of antibody production.

Considerations for Potential Applications of the AOP (optional)

CN is expressed in T cells as well as other types of immune cells like B cells and dendritic cells. CNIs suppress many kinds of immune functions leading to increased susceptibility to infections and decreased hyper immune reactions such as rejection and graft versus host disease (GVHD). Among these, TDAR is considered to be the most important endpoint of immunotoxicity, because T cells, B cells, and antigen-presenting cells such as dendritic cells are involved in inducing and developing of TDAR. Thus, changes in any of these immune cell populations can influence TDAR.

Moreover, when evaluating the immunotoxicity of pharmaceuticals, the ICH S8 immunotoxicity testing guideline recommends that TDAR be evaluated whenever the target cells of immunotoxicity are not clear based on pharmacology and findings in standard toxicity studies.

The present AOP could be used to predict whether or not a compound that potentially acts on T cells could also affect TDAR. On the other hand, it would be inappropriate to use the present AOP as an alternative to TDAR measurement in the ICH S8 immunotoxicity testing guideline.

References

- Alessiani, M., Kusne, S., Martin, M., Jain, A., Abu-Elmagd, K., Moser, J., Todo, S., Fung, J. and Starzl, T. (1991). Transplantation proceedings 23 (1 Pt 2): 1501-3.
- Antiga, E., Volpi, W., Torchia, D., Fabbri, P. and Caproni, M. (2011). Clinical and experimental dermatology 36 (3): 235-41.
- Beals, C.R., Clipstone, N.A., Ho, S.N. and Crabtree, G.R. (1997). Genes & development 11 (7): 824-34.
- Bhattacharyya, S., Deb, J., Patra, A.K., Thuy Pham, D.A., Chen, W., Vaeth, M., Berberich-Siebelt, F., Klein-Hessling, S., Lamperti, E.D., Reifenberg, K., Jellusova, J., Schweizer, A., Nitschke, L., Leich, E., Rosenwald, A., Brunner, C., Engelmann, S., Bommhardt, U., Avots, A., Müller, M.R., Kondo, E. and Serfling, E. (2011). The Journal of experimental medicine 208 (4): 823-39.
- Bierer, B.E., Holländer, G., Fruman, D. and Burakoff, S.J. (1993). Current opinion in immunology 5 (5): 763-73.
- Boussiotis, V.A., Nadler, L.M., Strominger, J.L. and Goldfeld, A.E. (1994).Proceedings of the National Academy of Sciences of the United States of America 91 (15): 7007-11.
- Bram, R.J., Hung, D.T., Martin, P.K., Schreiber, S.L. and Crabtree, G.R. (1993). Molecular and cellular biology 13 (8): 4760-9.
- Cameron, A.M., Nucifora, F.C. Jr., Fung, E.T., Livingston, D.J., Aldape, R.A., Ross, C.A. and Snyder, S.H. (1997). The Journal of biological chemistry 272 (44): 27582-8.
- Chung, B.H., Kim, K.W., Yu, J.H., Kim, B.M., Choi, B.S., Park, C.W., Kim, Y.S., Cho, M.L. and Yang, C.W. (2014). Transplant immunology 30 (4): 159-67.
- Cohan, V.L., Undem, B.J., Fox, C.C., Adkinson, N.F. Jr., Lichtenstein, L.M. and Schleimer, R.P. (1989). The American review of respiratory disease 140 (4): 951-4.

- Conboy, I.M., Manoli, D., Mhaiskar, V., and Jones, P.P. (1999). Proceedings of the National Academy of Sciences of the United States of America 96 (11):6324-9.
- Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Journal of immunology 160 (6): 2579-89.
- Ekberg, H., Tedesco-Silva, H., Demirbas, A., Vítko, S., Nashan, B., Gürkan, A., Margreiter, R., Hugo, C., Grinyó, J.M., Frei, U., Vanrenterghem, Y., Daloze, P. and Halloran, P.F.; ELITE-Symphony Study. (2007). The New England journal of medicine 357 (25): 2562-75.
- Ekberg, H., Bernasconi, C., Tedesco-Silva, H., Vítko, S., Hugo, C., Demirbas, A., Acevedo, R.R., Grinyó, J., Frei, U., Vanrenterghem, Y., Daloze, P. and Halloran, P. (2009). American journal of transplantation 9 (8): 1876-85.
- Flanagan, W.M., Corthésy, B., Bram, R.J. and Crabtree, G.R. (1991). Nature 352 (6338): 803-7.
- Foletta, V.C., Segal, D.H. and Cohen, D.R. (1998). Journal of leukocyte biology 63 (2): 139-52.
- Fruman, D.A., Bierer, B.E., Benes, J.E., Burakoff, S.J., Austen, K.F. and Katz, H.R. (1995). Journal of immunology 154 (4): 1846-51.
- Fung, J., Abu-Elmagd, K., Jain, A., Gordon, R., Tzakis, A., Todo, S., Takaya, S., Alessiani, M., Demetris, A., Bronster, O., Martin, M., Mieles, L., Selby, R., Reyes, J., Doyle, H., Stieber, A., Casavilla, A. and Starzl, T. (1991). Transplantation proceedings 23 (6): 2977-83.
- Glynne, R., Akkaraju, S., Healy, J.I., Rayner, J., Goodnow, C.C. and Mack, D.H. (2000). Nature 403 (6770): 672-6.
- Goldfeld, A.E., Flemington, E.K., Boussiotis, V.A., Theodos, C.M., Titus, R.G., Strominger, J.L. and Speck, S.H. (1992). Proceedings of the National Academy of Sciences of the United States of America 89 (24): 12198-201.
- Goldfeld, A. E., Tsai, E., Kincaid, R., Belshaw, P. J., Schrieber, S. L., Strominger, J. L. and Rao, A. (1994). Journal of experimental medicine. 180(2): 763-768.
- Heidt, S., Roelen, D. L., Eijsink, C., Eikmans, M., van Kooten, C., Claas, F. H. and Mulder, A. (2010). Clinical and experimental immunology. 159(2): 199-207.
- Hiroi, J., Sengoku, T., Morita, K., Kishi, S., Sato, S., Ogawa, T., Tsudzuki, M., Matsuda, H., Wada, A. and Esaki, K. (1998). Japanese journal of pharmacology. 76(2): 175-183.
- Hultsch, T., Albers, M. W., Schreiber, S.L. and Hohman, R. J. (1991). Proceedings of the national academic science of the United States of America. 14: 6229-6233.
- Imai, A., Sahara, H., Tamura, Y., Jimbow, K., Saito, T., Ezoe, K., Yotsuyanagi, T. and Sato, N. (2007). European journal of immunology. 37(7): 1730-1738.
- Jain, J., McCaffrey, P. G., Valge-Archer, V. E. and Rao, A. (1992). Nature. 356(6372): 801-804.
- Jain, J., Miner, Z. and Rao, A. (1993). Journal of immunology. 151(2): 837-848.
- Jennings, C., Kusler, B. and Jones, P. P. (2009). Innate immunity. 15(2): 109-120.
- Kang, Y. J., Kusler, B., Otsuka, M., Hughes, M., Suzuki, N., Suzuki, S., Yeh, W. C., Akira, S., Han, J. and Jones, P. P. (2007). Journal of immunology. 179(7): 4598-4607.
- Kang, C. B., Hong, Y., Dhe-Paganon, S. and Yoon, H. S. (2008). Neurosignals. 16: 318-325.
- Kim, T., Kim, N. and Kang, H. J. (2010). Journal of leukocyte biology. 88:1089-1097.
- Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., Goto, T., Okuhara, M., Kohsaka, M. and Aoki, H. (1987a). Journal of antibiotics. 40(9): 1256-1265.
- Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Imanaka, H. (1987b). Journal of antibiotics. 40(9): 1249-1255.
- Klee, C. B., Draetta, G. F. and Hubbard, M. J. (1988). Advances in enzymology and related areas of molecular biology. 61:149-200.
- Lee, Y. R., Yang, I. H., Lee, Y. H., Im, S. A., Song, S., Li, H., Han, K., Kim, K., Eo, S. K. and Lee, C. K. (2005). Blood. 105(10): 3951-3955.
- Liu, J., Farmer, J. D. Jr., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991). Cell. 66(4): 807-815.
- Liu, J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20J%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Albers, M. W (https://www.ncbi.nlm.nih.gov/pubmed/?term=Albers%20MW%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Wandless, T. J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Wandless%20TJ%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Luan, S (https://www.ncbi.nlm.nih.gov/pubmed/?term=Luan%20S%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Alberg, D. G (https://www.ncbi.nlm.nih.gov/pubmed/?term=Alberg%20DG%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Belshaw, P. J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Belshaw%20PJ%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Cohen, P (https://www.ncbi.nlm.nih.gov/pubmed/?term=Cohen%20P%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., MacKintosh, C (https://www.ncbi.nlm.nih.gov/pubmed/?term=MacKintosh%20C%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Klee, C. B (https://www.ncbi.nlm.nih.gov/pubmed/?term=Klee%20CB%5BAuthor%5D&cauthor=true&cauthor_uid=1373650). and Schreiber, S.L (https://www.ncbi.nlm.nih.gov/pubmed/?term=Schreiber%20SL%5BAuthor%5D&cauthor=true&cauthor_uid=1373650).. (1992). Biochemistry. (https://www.ncbi.nlm.nih.gov/pubmed/1373650) 31(16):3896-901.
- Liu, J. (1993). Immunology today. 14(6): 290-305.
- Macian, F. (2005). Nature reviews. Immunology. 5(6): 472-84.

- Magari, K., Miyata, S., Ohkubo, Y., Mutoh, S. and Goto, T. (2003). British journal of pharmacology. 139: 927-934.
- Matsuda, S., Koyasu, S. (2000). Tanpakushitsu kakusan koso. 45(11): 1823-1831.
- Meingassner, J.G. and Stütz, A. (1992). Journal of investigative dermatology 98(6): 851-5
- Nalesnik, MA., Todo, S., Murase, N., Gryzan, S., Lee, PH., Makowka, L., and Starzl, TE. (1987). Transplantation Proceedings 19(5 Suppl 6): 89-92.
- Panhans-Gross, A., Novak, N., Kraft, S., and Bieber, T. (2001). Journal of Allergy and Clinical Immunology 107(2): 345-52.
- Pirsch, JD., Miller, J., Deierhoi, MH., Vincenti, F., and Filo, RS. (1997). Transplantation 63(7): 977-83.
- Rao, A., Luo, C., and Hogan, PG. (1997). Annual Review of Immunology 15: 707-47.
- Sakuma, S., Kato, Y., Nishigaki, F., Sasakawa, T., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2000). British Journal of Pharmacology 130(7): 1655-63.
- Sakuma, S., Higashi, Y., Sato, N., Sasakawa, T., Sengoku, T., Ohkubo, Y., Amaya, T., and Goto, T. (2001a). International Immunopharmacology 1(6): 1219-26.
- Sakuma, S., Kato, Y., Nishigaki, F., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2001b). International Immunopharmacology 1(4): 749-57.
- Sasakawa, Y., Sakuma, S., Higashi, Y., Sasakawa, T., Amaya, T., and Goto, T. (2000). European Journal of Pharmacology 403(3): 281-8.
- Sasaki, T., Nakamura, W., Inokuma, S., and Matsubara, E. (2015). Journal of Clinical Rheumatology Feb 3.
- Schreiber, SL., and Crabtree, GR. (1992). Immunology Today 13(4): 136-42.
- Serfling, E., Berberich-Siebelt, F., Chuvpilo, S., Jankevics, E., Klein-Hessling, S., Twardzik, T., and Avots, A., (2000). Biochimica et Biophysica Act 1498 (1): 1-18.
- Siekierka, JJ., Hung, SH., Poe, M., Lin, CS., and Sigal, NH. (1989a). Nature 341(6244): 755-57.
- Siekierka, JJ., Staruch, MJ., Hung, SH., and Sigal, NH. (1989b). Journal of immunology 143(5): 1580-3.
- Siekierka, JJ., Wiederrecht, G., Greulich, H., Boulton, D., Hung, SH., Cryan, J., Hodges, PJ., and Sigal, NH. (1990). Journal of Biological Chemistry 265(34): 21011-5.
- Sonoda, T., Takahara, S., Takahashi, K., Uchida, K., Ohshima, S., Toma, H., Tanabe, K., Yoshimura, N.; Japanese Tacrolimus Study Group. (2003). Transplantation 75(2): 199-204.
- Standaert, RF., Galat, A., Verdine, GL., and Schreiber, SL. (1990). Nature 346(6285): 671-4.
- Tamura, F., Masuhara, A., Sakaida, I., Fukumoto, E., Nakamura, T., and Okita, K. (1998). Journal of Gastroenterology and Hepatology 13(7): 703-8.
- Ulrich, P., Paul, G., Perentes, E., Mahl, A., and Roman D. (2004). Toxicology Letters 149(1-3): 123-31.
- Vacher-Coponat, H., Brunet, C., Moal, V., Loundou, A., Bonnet, E., Lyonnet, L., Ravet, S., Sampol-Manos, E., Sampol, J., Berland, Y., George, FD., and Paul, P. (2006). Transplantation 82(4): 558-66.
- Vandewalle, A., Tourneur, E., Bens, M., Chassin, C., and Werts, C. (2014). Cell Communication and Signaling 12: 8
- Weiwad, M., Edlich, F., Kilka ,S., Erdmann, F., Jarczowski, F., Dorn, M., Moutty, M.C. and Fischer, G. (2006). Biochemistry 45(51): 15776-84.
- Wicker, L.S., Boltz, R.C. Jr., Matt, V., Nichols. E.A., Peterson, L.B. and Sigal, N.H. (1990). European journal of immunology 20(10): 2277-83.
- Yoshimura, N., Matsui, S., Hamashima, T. and Oka, T. (1989). Transplantation 47(2): 356-9.
- Yoshino, T., Nakase, H., Honzawa, Y., Matsumura, K., Yamamoto, S., Takeda, Y., Ueno, S., Uza, N., Masuda, S., Inui, K. and Chiba, T. (2010). Inflammatory bowel disease. 16(12): 2022-33
- Zhang, B.W., Zimmer, G., Chen, J., Ladd, D., Li, E., Alt, F.W., Wiederrecht, G., Cryan, J., O'Neill, E.A., Seidman, C.E., Abbas, A.K. and Seidman, J.G.. (1996). Journal of experimental medicine 183(2): 413-20.
- Zhu, J. and McKeon, F. (1999). Nature. 398(6724): 256-60.
- de Paulis, A., Cirillo, R., Ciccarelli, A., de Crescenzo, G., Oriente, A. and Marone, G. (1991). Journal of immunology 147(12): 4278-85.
- de Paulis, A., Stellato, C., Cirillo, R., Ciccarelli, A., Oriente, A. and Marone, G. (1992). Journal of investigative dermatology 99(6): 723-8.
- van Dieren, J.M., Lambers, M.E.H., Kuipers, E.J., Samsom, J.N., van der Woude, C.J. and Nieuwenhuis, E.E.S. (2010). Digestive diseases and sciences 55(9): 2514-19.
- van Lierop, P.P., de Haar, C., Lindenbergh-Kortleve, D.J., Simons-Oosterhuis, Y., van Rijt, L.S., Lambrecht, B.N., Escher, J.C., Samsom, J.N. and Nieuwenhuis, E.E. (2010). Inflammatory bowel disease 16(3): 442-51.
- Maruho Co.,Ltd. (2014) Drug interview form Protopic ointment 0.1% Revised 16th edition.
- Astellas Pharma Inc. (2014) Drug interview form Prograf capsules 0.5mg, 1mg, 5mg, granules 0.2mg, 1mg. Revised 34th edition
- Astellas Pharma Inc. (2014) Drug interview form Prograf capsules 0.5 mg, 1 mg, 5 mg, granules 0.2 mg, 1 mg. Revised 34th edition

- Fyjii Y., Gogi H., Takamura K., Sakuma A. and Goto T. Kisotorinsyo 31(8): 2693-2700 (in Japanese)
- Sengoku T., Morita K., Sato A., Sakuma S., Ogawa T., Hiroi J., Fujii T and Goto T. (1998) Folia Pharmacol. Jpn. (Nippon Yakurigaku Zasshi) 112, 221-232

Appendix 1

List of MIEs in this AOP

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

Short Name: Inhibition, Calcineurin Activity

Key Event Component

Process	Object	Action
binding	FK506-binding protein 15	increased
binding	FKBP12 (Arabidopsis thaliana)	increased

AOPs Including This Key Event

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

Stressors

Name
Tacrorimus
Cyclosporin

Biological Context

Level of Biological Organization
Molecular

Organ term

Organ term
immune system

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

CN is broadly distributed in 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 CnA isoforms from different organisms (Kincaid. 1996).

As for immunophilins, of which complexes inhibit the CN activity, 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 living beings have PPIase activity (Wang P et al. 2005).

However, inhibition of CN phosphatase activity through immunophilin-CNI complex has been reported at least in rodents and humans.

Key Event Description

Calcineurin (CN) 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.

Immunophilins are a general class of proteins that exhibit peptidyl-propyl isomerase (PPIase) activity (Barik. 2006) and an immunophilin-CN inhibitor (CNI) complex such as FKBP12- FK506 and cyclophilin-CsA binds directly to CnA in the cell, causing steric hindrance of substrate binding to CN, which inhibits the phosphatase activity of CN without any contribution of PPIase activity (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. CN, 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 CN by the FKBP12-FK506 complex are conducted. (Bram et al. 1993). Phosphatase activity of CN in the presence of cyclosporin A (CsA) and cyclophilin can also be determined in the manner described above.

Immunophilin-CNI complexes directly inhibit phosphatase activity of CN, therefore, as a surrogate measurement of the CN activity, the binding of CsA 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).

References

1. Barik, S. (2006). Immunophilins: for the love of proteins. Cellular and Molecular Life Sciences 63(24): 2889-900.
2. Bierer, B.E., Holländer, G., Fruman, D. and Burakoff, S.J. (1993). Cyclosporin A and FK506: molecular mechanisms of immunosuppression and probes for transplantation biology. Current opinion in immunology 5 (5): 763-73.
3. Bram, R.J., Hung, D.T., Martin, P.K., Schreiber, S.L. and Crabtree, G.R. (1993). Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcimeurin binding and cellular location. Molecular and cellular biology 13 (8): 4760-9.
4. Cameron, A.M., Nucifora, F.C. Jr., Fung, E.T., Livingston, D.J., Aldape, R.A., Ross, C.A. and Snyder, S.H. (1997). FKBP12 binds the inositol 1, 4, 5-trisphosphate receptor at leucine-proline (1400-1401) and anchors calcineurin to this FK506-like domain. The Journal of biological chemistry 272 (44): 27582-8.
5. Hultsch, T., Albers, M. W., Schreiber, S.L. and Hohman, R. J. (1991). Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. Proceedings of the national academic science of the United States of America. 14: 6229-6233.
6. Kang, C. B., Hong, Y., Dhe-Paganon, S. and Yoon, H. S. (2008). FKBP family proteins: immunophilins with versatile biological function. Neurosignals. 16: 318-325.
7. Kincaid, R..L. (1993). Calmodulin-dependent protein phosphatases from microorganisms to man. A study in structural conservatism and biological diversity. Adv Second Messenger Phosphoprotein Res. 1993;27:1-23.
8. Klee, C. B., Draetta, G. F. and Hubbard, M. J. (1988). Calcineurin. Advances in enzymology and related areas of molecular biology. 61:149-200.

9. Liu, J., Farmer, J. D. Jr., Lane, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 66(4): 807-815.
10. Liu, J. (1993). FK506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunology today. 14(6): 290-305.
11. Quesniaux VF, Schreier MH, Wenger RM, Hiestand PC, Harding MW, Van Regenmortel MH(1987). Cyclophilin binds to the region of cyclosporine involved in its immunosuppressive activity.
12. Panhans-Gross, A., Novak, N., Kraft, S., and Bieber, T. (2001). Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). Journal of Allergy and Clinical Immunology 107(2): 345-52.
13. Rao, A., Luo, C., and Hogan, PG. (1997). Transcription factors of the NFAT family: regulation and function. Annual Review of Immunology 15: 707-47.
14. Schreiber, SL., and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42.
15. Siekierka, JJ., Hung, SH., Poe, M., Lin, CS., and Sigal, NH. (1989a). A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 341(6244): 755-57.
16. Siekierka, JJ., Wiederrecht, G., Greulich, H., Boulton, D., Hung, SH., Cryan, J., Hodges, PJ., and Sigal, NH. (1990). The cytosolic-binding protein for the immunosuppressant FK-506 is both a ubiquitous an highly conserved peptidyl-prolyl cis-trans isomerase. Journal of Biological Chemistry 265(34): 21011-5.
17. Zhang, B.W., Zimmer, G., Chen, J., Ladd, D., Li, E., Alt, F.W., Wiederrecht, G., Cryan, J., O'Neill, E.A., Seidman, C.E., Abbas, A.K. and Seidman, J.G.. (1996). T cell responses in calcineurin A alpha-deficient mice. Journal of experimental medicine 183(2): 413-20.

List of Key Events in the AOP

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

Short Name: Interference, nuclear localization of NFAT

Key Event Component

Process	Object	Action
genetic interference	NFAT protein	increased

AOPs Including This Key Event

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

Stressors

Name
Tacrolimus
Cyclosporin

Biological Context

Level of Biological Organization
Molecular

Organ term

Organ term
immune system

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

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

Key Event Description

The nuclear factor of activated T cells (NFAT) is a substrate of calcineurin (CN) (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 are phosphorylated, which covers the NLS and leaves the NES exposed, so that NFAT localizes in cytoplasm. When SP motifs are dephosphorylated by activated CN, the NLS is exposed and the NES is covered, thereby promoting nuclear localization of NFAT (Matsuda and Koyasu 2000, Zhu and McKeon 1999). When T-cell activation takes place, T-cell–receptor-mediated stimulus increases the intracellular concentration of calcium and activates a regulatory subunit (CnB), which subsequently induces a catalytic subunit (CnA) phosphatase activation, leading to dephosphorylation of NFAT followed by nuclear localization. CN inhibitor -immunophilin complexes inhibit CN phosphatase activation, thereby interfering with NFAT nuclear localization (Bhattacharyya et al.2011).

How it is Measured or Detected

Nuclear translocation of NFAT can be tested by imaging flowcytometer, in which lymphocytes are treated with fluorescence-labeled anti-NFAT antibody and DAPI (nuclear stain) and intracellular distribution of NFAT is analyzed by imaging flowcytometry with image analysis (Maguire O et al. 2013).

Interference with translocation of NFAT to the nucleus can be detected using a gel mobility shift assay of nuclear or cytoplasmic extracts electrophoresed with end-labeled NFAT-binding site from human IL-2 enhancer (Flanagan et al. 1991).

References

1. Rao, A., Luo, C., and Hogan, PG. (1997). Transcription factors of the NFAT family: regulation and function. Annual Review of Immunology 15: 707-47.
2. Beals, C.R., Clipstone, N.A., Ho, S.N. and Crabtree, G.R. (1997). Nuclear localization of NF-ATc by a calcineurin-dependent, cyclosporin-sensitive intramolecular interaction. Genes & development 11 (7): 824-34.
3. Zhu, J. and McKeon, F. (1999). NF-AT activation requires suppression of Crm1-dependent export by calcineurin. Nature. 398(6724): 256-60.
4. Serfling, E., Berberich-Siebelt, F., Chuvpilo, S., Jankevics, E., Klein-Hessling, S., Twardzik, T., and Avots, A., (2000). The role of NF-AT transcription factors in T cell activation and differentiation. Biochimica et Biophysica Act 1498 (1): 1-18.
5. Matsuda, S., Koyasu, S. (2000). A second target of cyclosporin A and FK506. Tanpakushitsu kakusan koso. 45(11): 1823-1831.
6. Bhattacharyya, S., Deb, J., Patra, A.K., Thuy Pham, D.A., Chen, W., Vaeth, M., Berberich-Siebelt, F., Klein-Hessling, S., Lamperti, E.D., Reifenberg, K., Jellusova, J., Schweizer, A., Nitschke, L., Leich, E., Rosenwald, A., Brunner, C., Engelmann, S., Bommhardt, U., Avots, A., Müller, M.R., Kondo, E. and Serfling, E. (2011). NFATc1 affects mouse splenic B cell function by controlling the calcineurin-NFAT signaling network. The Journal of experimental medicine 208 (4): 823-39.
7. 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.
8. Maguire O., Tornatore K.M., O'Loughlin K.L., Venuto R.C., Minderman H.(2013). Nuclear translocation of nuclear factor of activated T cells (NFAT) as a quantitative pharmacodynamic parameter for tacrolimus.

Event: 981: Reduction, NFAT/AP-1 complex formation (https://aopwiki.org/events/981)

Short Name: Reduction, NFAT/AP-1 complex formation

Key Event Component

Process	Object	Action
cytokine production involved in inflammatory response	NFAT activation molecule 1	decreased
cell activation		increased

AOPs Including This Key Event

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

Stressors

Name
Tacrolimus
Cyclosporin

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
T cell

Organ term

Organ term
immune system

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

CN-NFAT system functionality is common among mammalian species, including humans and rodents. It is also possible that FK506-induced interference with NFAT/AP-1 complex formation at the promoter site of the IL-2 gene is common among mammalian T cells, including those of humans and rodents (Flanagan et al. 1991).

Key Event Description

Activated nuclear factor of activated T cells (NFAT) that has localized to the nucleus binds cooperatively at the site of the Interleukin-2 (IL-2) promoter with activator protein-1 (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). Interfered nuclear localization of NFAT, induced by FK506, hinders the formation of the functional NFAT/AP-1 complexes necessary to binding at the site of IL-2 promoters (Flanagan et al. 1991).

NFAT is known to bind cooperatively at the promoters of Interleukin-4 (IL-4) and other T-cell cytokines as well as that of IL-2 (Macian et al. 2005).

How it is Measured or Detected

Reductions in NFAT/AP-1 complex formation can be detected using a gel shift assay to test nuclear extracts from either stimulated or unstimulated Ar-5 T cells with radio-labelled NFAT binding oligonucleotide from murine IL-2 promoter. Anti-Fos and anti-Jun antibodies are used to examine NFAT/AP-1 complex formation (Jain et al. 1992).

References

1. 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.
2. Jain, J., McCaffrey, P. G., Valge-Archer, V. E. and Rao, A. (1992). Nuclear factor of activated T cells contains Fos and Jun. Nature. 356(6372): 801-804.
3. Jain, J., Miner, Z. and Rao, A. (1993). Analysis of the preexisting and nuclear forms of nuclear factor of activated T cells. Journal of immunology. 151(2): 837-848.
4. Macian, F. (2005). NFAT proteins: key regulators of T-cell development and function. Nature reviews. Immunology. 5(6): 472-84.
5. Schreiber, SL., and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42.

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

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

Key Event Component

Process	Object	Action
interleukin-2 production	interleukin-2	decreased
interleukin-4 production	interleukin-4	decreased

AOPs Including This Key Event

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

Stressors

Name
Tacrolimus
Cyclosporin

Biological Context

Level of Biological Organization
Cellular

Organ term

Organ term
immune system

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

CNIs 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, CNIs (FK506 and CsA) 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).

CNIs (FK506 and CsA) exhibit suppression of IL-2 production induced from mixed lymphocyte reactions in mice and humans (Kino, T et al. 1987a).

These facts indicate that CN-NFAT system-mediated suppression of cytokines is commonly found in humans and mice.

Key Event Description

Production of T cell cytokines including Interleukin-2 (IL-2) and interleukin-4 (IL-4) is regulated by NFAT/AP-1 complexes. Activated NFAT/AP-1 complex that bind at the site of the IL-2 and IL-4 promoters, thereby induces transcription of IL-2 (Jain et al. 1993). For IL-2, NFAT proteins are necessary for IL-2 gene expression and cooperation of NFAT with AP-1 is required for IL-2 gene transcription. For IL-4, At least five different NFAT sites have been described in the IL-4 promoter with at least three of them being composite sites binding NFAT and AP-1 (Macián et al. 2001).

Calcineurin inhibitors (CNIs) such as FK506 and cyclosporin A (CsA) hinder the formation of the functional NFAT/AP-1 complexes by interfering with NFAT nuclear localization (Flanagan et al. 1991). Reduced binding of NFAT/AP-1 complexes at the promoter site of the IL-2 gene lowers the transcription of the mRNA of IL-2 and the following cytokine production.

Transcription of IL-4 is also inhibited by CNIs in the same manner as IL-2 (Dumont et al. 1998).

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 3,3',5,5'-tetramethylbenzidine (TMB) substrate. ELISA plates were scanned in a Molecular Devices UVmax plate reader (Menlo Park, CA), using SOFT max 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

1. Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.
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.
3. Jain, J., McCaffrey, P. G., Valge-Archer, V. E. and Rao, A. (1992). Nuclear factor of activated T cells contains Fos and Jun. Nature. 356(6372): 801-804.
4. Jain, J., Miner, Z. and Rao, A. (1993). Analysis of the preexisting and nuclear forms of nuclear factor of activated T cells. Journal of immunology. 151(2): 837-848.
5. Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., Goto, T., Okuhara, M., Kohsaka, M. and Aoki, H. (1987a). FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. Journal of antibiotics. 40(9): 1256-1265.
6. Macián, F., López-Rodríguez, C. and Rao, A. (2001). Partners in transcription: NFAT and AP-1. Oncogene. 20(19): 2476-89.
7. Sakuma, S., Higashi, Y., Sato, N., Sasakawa, T., Sengoku, T., Ohkubo, Y., Amaya, T., and Goto, T. (2001a). Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids). International Immunopharmacology 1(6): 1219-26.
8. Schreiber, SL., and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42.

List of Adverse Outcomes in this AOP

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

Short Name: Impairment, T-cell dependent antibody response

Key Event Component

Process	Object	Action
Immunosuppression		increased

AOPs Including This Key Event

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

Stressors

Name
Tacrolimus
Cyclosporin

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

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

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

CNI induced impairment of TDAR is demonstrated with rodent studies. That is, oral administration of FK506 or CsA to mice for 4 days impaired the response of plaque-forming cells (PFC) in splenocytes after intravenous immunization with sheep erythrocytes (Kino et al. 1987). Likewise, oral administration of FK506 to rats over a four-week period reduced production of both anti-KLH(keyhole limpet hemocyanin)-IgG and IgM antibodies after subcutaneous immunization with KLH (Ulrich et al. 2004). As for humans, in vitro experiments showed that treatment with FK506 or CsA of peripheral blood mononuclear cells from blood-bank donors suppressed the production of IgM and IgG antibodies specific to T-cell–dependent antigens. (Heidt et al, 2009) Also, in SKW6.4 cells (IL-6–dependent, IgM-secreting, human B-cell line) cultures, FK506 or cyclosporin suppressed the production of IgM antibodies in the presence of T-cell activation. (Sakuma et al. 2001b) Considering that FKF506 and CsA reduce T cell-derived cytokines including IL-2 and IL-4, these findings strongly suggest that impairment of TDAR following reduced production of such cytokines occurs at least in common among humans and rodents.

Key Event Description

Antibody production to T-cell–dependent antigens is established through the coordination of B cells, antigen-presenting cells as well as T-cell–derived cytokines, which stimulate B cells to proliferate and differentiate. T-cell–dependent antibody response (TDAR) might be altered if any of these cell populations is affected.

IL-2 stimulates B cells to proliferate through surface IL-2 receptors. IL-4 stimulates B-cells to proliferate, to switch immunoglobulin classes, and to differentiate into plasma and memory cells. Suppressing the production of these B-cell–related cytokines appears to impaire TDAR, as seen in the result of FK506 treatment (Heidt et al, 2009).

IL-2 and IL-4 are produced and secreted by helper T cells and play important roles in the development of TDAR. IL-4 affects maturation and class switching of B cells as well as proliferation, both of which induces/enhances T cell dependent antibody production. IL-2 promotes differentiation of B cells through IL-2 stimulates differentiation of the activated T cell into T cell called Th2 cell. Therefore, suppressed production of IL-2 and IL-4 impairs TDAR (Alberts et al. 2008).

How it is Measured or Detected

TDAR could be examined in vivo and in vitro.

In vivo studies of antigen-specific antibodies are usually performed by measuring serum antibody levels with ELISA or with a plaque-forming cell (PFC) assay.

- Rats were repeatedly administered FK506 orally for 4 weeks and immunized with Keyhole limpet hemocyanin (KLH), after which the serum was examined for T-cell–dependent, antigen-specific, IgM and IgG levels using a Sandwich ELISA kit (Ulrich et al. 2004).
- Mice were repeatedly administered CNI including FK506 and CsA orally for 4 days and immunized with sheep red blood cells (SRBC), after which spleen cells were examined using a PFC assay (Kino et al. 1987).

For in vitro studies, total IgM and IgG levels in culture supernatant are often measured after polyclonal T-cell activation rather than measuring antigen stimulation in immune cell cultures.

- T cells and B cells isolated from human peripheral blood mononuclear cells (PBMC) were co-cultured with a 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. Treatment with FK506 or CsA reduced the levels of IgM and IgG at the concentrations of 0.3 and 1.0 ng/mL or 50 and 100 ng/mL (Heidt et al, 2009).
- SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) 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. FK506 or CsA reduced the levels of IgM at the concentrations of 0.01 to 100 ng/mL or 0.1 to 1000 ng/mL (Sakuma et al. 2001b).
- In order to examine 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 (Dumont et al. 1998).

Regulatory Significance of the AO

ICH Harmonised tripartite guideline Immunotoxicity studies for human pharmaceuticals S8.

References

1. Alberts, B., Johnson, A., Lewis, L., Raff, M., Roberts, K. and Walter, P. (2008). Molecular Biology of the Cell. 5th ed., Garland Science, New York. 1539-1601
2. Heidt, S., Roelen, D. L., Eijsink, C., Eikmans, M., van Kooten, C., Claas, F. H. and Mulder, A. (2010). Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. Clinical and experimental immunology. 159(2): 199-207.
3. Sakuma, S., Kato, Y., Nishigaki, F., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2001b). Effects of FK506 and other immunosuppressive anti-rheumatic agents on T cell activation mediated IL-6 and IgM production in vitro. International Immunopharmacology 1(4): 749-57.
4. Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Imanaka, H. (1987). FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. Journal of antibiotics. 40(9): 1249-1255.
5. Ulrich, P., Paul, G., Perentes, E., Mahl, A., and Roman D. (2004). Validation of immune function testing during a 4-week oral toxicity study with FK506. Toxicology Letters 149(1-3): 123-31.
6. Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

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

AOPs Referencing Relationship

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

CN is broadly distributed throughout the body, and the structure of CnA and CnB is highly conserved from yeasts to humans (Kincaid. 1993).

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

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).

These facts indicate that CN and immunophilins are conserved among animals and plants although they show multiple physiological functions.

In addition, CNI/immunophilin complex-induced inhibition of CN phosphatase activity resulting in suppression of immune responses is found in humans and mice.

Key Event Relationship Description

The phosphatase activity of calcineurin (CN) is known to be inhibited by CN inhibitors (CNIs) such as FK506 and cyclosporin A (CsA) through the formation of complexes with immunophilins.

Immunophilins of FK506-binding protein (FKBP) and cyclophilin bind with CNIs FK506 and CsA to form complexes, which inhibit CN 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 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 peptidyl propyl isomerase (PPIase) activity, but the PPIase activity and the inhibition of activity that they indicate are unrelated to CN regulation.

CN 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.

CNI-immunophilin complexes such as FK506/FKBP complexes and cyclophilin/CsA complexes bind directly to CnA in the cell, causing steric hindrance of substrate binding to CN, which in turn inhibits phosphatase activity of CN (Schreiber and Crabtree 1992, Liu et al. 1993, Bierer et al. 1993, Bram et al. 1993, Rao et al. 1997, Liu et al. 1991).

The nuclear factor of activated T cells (NFAT) is a substrate of CN (Rao et al. 1997).

When T-cell activation takes place, T-cell–receptor-mediated stimulus increases the intracellular concentration of calcium and activates CnB, which subsequently induces CnA phosphatase activation, leading to dephosphorylation of NFAT. In that process, . dephosphorylated SP motifs exposes nuclear localization signal (NLS) and covers nuclear export signal (NES), thereby promoting nuclear localization of NFAT (Matsuda and Koyasu 2000, Zhu and McKeon 1999).

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

Evidence Supporting this KER

Biological Plausibility

The molecular structures and functions of CN and NFAT are evident based on sufficient scientific findings as mentioned above The known mechanisms for inhibition of CN phosphatase activity by FK506, CsA, or other CNIs are initiated by the formation of complexes with their respective immunophilin species. Immunophilins are general classes of proteins that exhibit PPIase activity, but modification of these functions is unrelated to inhibition of CN activity and thus thought to arise in the molecular structure of the complexes (Schreiber and Crabtree 1992, Liu et al. 1993, Bierer et al. 1993, Bram et al. 1993, Rao et al. 1997, Liu et al. 1991).

As mentioned above, inhibition of CN phosphatase activity interferes with the dephosphorylation of NFAT, which leads to the suppression of its nuclear localization.

Empirical Evidence

Much experimental data is available that supports the inhibition of CN activity induced by CNI/immunophilin complexes, which subsequently suppress nuclear localization of NFAT. In addition, CN phosphatase activity is inhibited by 24 hours treatment with CNI of FK506 and CsA with IC50 values of 0.5 and 5 nM, respectively (Fruman et al.1992).

Also, concentration-dependent reduction of in vitro nuclear localization of NFAT was evident using imaging flowcytometry at the maximum concentration of 1 μM with minimal concentration of 0.1nM (Jurkat human T cell line) or 10nM (T cells from whole blood) after 2 hours treatment of tacrolimus (Maguire et al. 2013). Interference with translocation of NFAT to the nucleus is also detected using gel mobility shift assay to test nuclear extracts and cytoplasmic extracts, in which the examined concentration of FK506 was 10ng/mL (Flanagan et al. 1991).

These findings show that dose responses and temporality of MIE and KE1 seem to be the same.

Uncertainties and Inconsistencies

CN and NFAT are expressed in T cells and other immune cells including B cells, DC, and NKT cells and related to cytokine productions from these immune cells. Also, expression of IL-2 receptors (IL-2R) in DCs are lowered due to the inhibition of CN phosphatase activity by CNI

treatment. Of these, reduced production of IL-2 and IL-4 from T cells plays a major role in suppression of TDAR due to lower proliferation, differentiation, and class switching of B cells. There have been no reports of CNI-induced reduction of cytokines other than IL-2 and IL-4 or reduced expression of IL-2R resulting in TDAR suppression.

FKBP12, a specific immunophilin that binds with FK506, is also an accessory molecule that binds to IP3 and Ryanodine receptors, both of which occur in Ca channels located on the membrane of the endoplasmic reticulum and participate in the regulation of intracellular Ca concentration. When binding with FK506, FKBP12 leaves these receptors to increase the influx of Ca2+ from the endoplasmic reticulum to cytoplasm, which should increase CN activity. Treatment with FK506, however, suppresses NFAT nuclear localization. In addition, FKBP12-knock out mice show no changes in immune function, including T-cell function. These facts suggest that the inhibition of CN-NFAT systems induced by FK506 treatment results from direct inhibition of CN phosphatase activity by FK506/FKBP12 complexes and not by affecting Ryanodine and IP3 receptors associated with FKBP12.

Quantitative Understanding of the Linkage

Response-response relationship
MIE:

Dose-response analysis of the effects of FK506 on CN phosphatase activity in mast cell-derived KiSVMC4W cells transfected with human FKBP12 cDNA showed that increased expression of FKBP12 resulted in a greater than ten-fold increase in sensitivity to FK506-mediated inhibition, as indicated by an IC50 value of roughly 2 nM with linear inverse dose-response curve after 1 hour incuvation (Fruman et al.1995). Another phosphatase assay showed that FK506 inhibition of CN activity was concentration-dependent reverse sigmoidal and that IC50 values for CN inhibition were approximately 0.5 nM for FK 506 and 5 nM for CsA after 1 hour culture (Fruman et al.1992).

KE1:

Dose-dependent interference with nuclear translocation of NFAT1 was observed with increasing CNI concentrations from 0.1 nM (Jurkat human T cells) up to 1 μM (1000 nM) using imaging flowcytometer. Higher concentrations induced cellular toxicity and resulted in cell death. Dose-dependent interference of nuclear NFAT1 translocation per CN inhibition was also observed in CD4+ T cells from healthy donors, again at maximal concentrations of 1 μM with minimum concentration of 10nM (Maguire et al. 2013).

There have been no literature available to compare directly the dose response of inhibition of CN phosphatase activity with that of nuclear translocation of NFAT; however, the concentration ranges of CNIs for inhibition of CN phosphatase activity and nuclear translocation of NFAT seem to be the same range.

Time-scale
Inhibition of CN phosphatase activity was examined after 1 hour culture of T cells (Fruman et al.1995, Fruman et al.1992), and inhibition of nuclear translocation of NFAT was measured by imaging flowcytometry after 2 hour culture of T cells with CNI (Maguire et al. 2013).

Known modulating factors
(To be described)

Known Feedforward/Feedback loops influencing this KER
(To be described)

References

1. Barik, S. (2006). Immunophilins: for the love of proteins. Cellular and Molecular Life Sciences 63(24): 2889-900.
2. Bierer, B.E., Holländer, G., Fruman, D. and Burakoff, S.J. (1993). Cyclosporin A and FK506: molecular mechanisms of immunosuppression and probes for transplantation biology. Current opinion in immunology 5 (5): 763-73.
3. Bram, R.J., Hung, D.T., Martin, P.K., Schreiber, S.L. and Crabtree, G.R. (1993). Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcimeurin binding and cellular location. Molecular and cellular biology 13 (8): 4760-9.
4. 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.
5. Fruman, D. A., Klee, C. B., Bierer, B. E. and Burakoff, S. J. (1992). Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proceedings of the National Academy of Sciences of the United States of America. 89(9):3686-90.
6. Fruman, D. A., Bierer, B. E., Benes, J. E., Burakoff, S. J., Austen, K. F. and Katz, H. R. (1995). The complex of FK506-binding protein 12 and FK506 inhibits calcineurin phosphatase activity and IgE activation-induced cytokine transcripts, but not exocytosis, in mouse mast cells. Journal of Immunology.154(4):1846-51.
7. Hultsch, T., Albers, M. W., Schreiber, S.L. and Hohman, R. J. (1991). Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. Proceedings of the national academic science of the United States of America. 14: 6229-6233.
8. Kang, C. B., Hong, Y., Dhe-Paganon, S. and Yoon, H. S. (2008). FKBP family proteins: immunophilins with versatile biological functions. Neurosignals. 16: 318-325.
9. Kincaid, R .L. (1993). Calmodulin-dependent protein phosphatases from microorganisms to man. A study in structural conservatism and biological diversity. Adv Second Messenger Phosphoprotein Res. 27:1-23.
10. Klee, C. B., Draetta, G. F. and Hubbard, M. J. (1988). Calcineurin. Advances in enzymology and related areas of molecular biology. 61:149-200.
11. Liu, J., Farmer, J. D. Jr., Lane, W. S., Friedman, J., Weissman, I. and Schreiber, S. L. (1991). Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell. 66(4): 807-815.
12. Liu, J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20J%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Albers, M. W (https://www.ncbi.nlm.nih.gov/pubmed/?term=Albers%20MW%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Wandless, T. J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Wandless%20TJ%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Luan, S (https://www.ncbi.nlm.nih.gov/pubmed/?term=Luan%20S%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Alberg, D. G

(https://www.ncbi.nlm.nih.gov/pubmed/?term=Alberg%20DG%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Belshaw, P. J (https://www.ncbi.nlm.nih.gov/pubmed/?term=Belshaw%20PJ%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Cohen, P (https://www.ncbi.nlm.nih.gov/pubmed/?term=Cohen%20P%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., MacKintosh, C (https://www.ncbi.nlm.nih.gov/pubmed/?term=MacKintosh%20C%5BAuthor%5D&cauthor=true&cauthor_uid=1373650)., Klee, C. B (https://www.ncbi.nlm.nih.gov/pubmed/?term=Klee%20CB%5BAuthor%5D&cauthor=true&cauthor_uid=1373650). and Schreiber, S.L (https://www.ncbi.nlm.nih.gov/pubmed/?term=Schreiber%20SL%5BAuthor%5D&cauthor=true&cauthor_uid=1373650).. (1992). Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Biochemistry. (https://www.ncbi.nlm.nih.gov/pubmed/1373650) 31(16):3896-901.

13. Liu, J. (1993). FK506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunology today. 14(6): 290-305.
14. Maguire O, Tornatore KM, O'Loughlin KL, Venuto RC and Minderman H. (2013) Nuclear translocation of nuclear factor of activated T cells (NFAT) as a quantitative pharmacodynamic parameter for tacrolimus. Cytometry A. 83(12):1096-104.
15. Matsuda, S., Koyasu, S. (2000). A second target of cyclosporin A and FK506. Tanpakushitsu kakusan koso. 45(11): 1823-1831.
16. Panhans-Gross, A., Novak, N., Kraft, S. and Bieber, T. (2001). Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). Journal of Allergy and Clinical Immunology 107(2): 345-52.
17. Rao, A., Luo, C. and Hogan, PG. (1997). Transcription factors of the NFAT family: regulation and function. Annual Review of Immunology 15: 707-47.
18. Schreiber, SL. and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42. >
19. Siekierka, JJ., Hung, SH., Poe, M., Lin, CS. and Sigal, NH. (1989). A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 341(6244): 755-57.
20. Siekierka, JJ., Wiederrecht, G., Greulich, H., Boulton, D., Hung, SH., Cryan, J., Hodges, PJ. and Sigal, NH. (1990). The cytosolic-binding protein for the immunosuppressant FK-506 is both a ubiquitous and highly conserved peptidyl-prolyl cis-trans isomerase. Journal of Biological Chemistry 265(34): 21011-5.
21. Wang, P. and Heitman, J. (2005) The cyclophilins. Genome Biology 6 (7):226.
22. Zhang, B.W., Zimmer, G., Chen, J., Ladd, D., Li, E., Alt, F.W., Wiederrecht, G., Cryan, J., O'Neill, E.A., Seidman, C.E., Abbas, A.K. and Seidman, J.G. (1996). T cell responses in calcineurin A alpha-deficient mice. Journal of experimental medicine 183(2): 413-20.
23. Zhu, J. and McKeon, F. (1999). NF-AT activation requires suppression of Crm1-dependent export by calcineurin. Nature. 398(6724): 256-60.

Relationship: 1017: Interference, nuclear localization of NFAT leads to Reduction, NFAT/AP-1 complex formation (https://aopwiki.org/relationships/1017)

AOPs Referencing Relationship

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	

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

CN-NFAT system functionality is common among mammalian species, including humans and rodents. It is also possible that FK506-induced interference with NFAT/AP-1 complex formation at the promoter site of the IL-2 gene is common among mammalian T cells, including those of humans and rodents (Flanagan et al. 1991).

Key Event Relationship Description

Activated (dephosphorylated) nuclear factor of activated T cells (NFAT) is translocated into the nucleus through the molecular changes of exposing nuclear localization signal (NLS) and concomitant masking of nuclear export signal (NES) due to dephosphorylation of the SP motifs of NFAT. (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) and induces transcription of IL-2 (Jain et al. 1993). 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.

Once CN phosphatase activity is inhibited, dephosphorylation of NFAT and subsequent nuclear localization of NFAT decreases, which results in a decrease of NFAT/AP-1 complex formation at the cytokine promoter sites (Rao et al. 1997).

Evidence Supporting this KER

Biological Plausibility
As has been mentioned, NFAT has NLS and NES among and adjacent to the N-terminal region rich in SP motifs, and once the SP region is dephosphorylated, the NLS is exposed and the NES is covered, which leads to translocation of NFAT into the nucleus (Matsuda and Koyasu 2000).

It is well known from the experiments using CN inhibitors (CNIs) that interference with the nuclear localization of NFAT in T cells leads to a reduction in the formation of NFAT/AP-1 complexes, thereby suppressing transcription of IL-2, IL-4, and a number of other cytokines (Maguire et al. 2013, Jain et al. 1992, Jain et al. 1993).

In contrast to T cells, B-cell receptor-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.

Expression of IL-2 receptors in dendritic cells and NKT cells is also reported to be regulated by this CN-NFAT system (Panhans-Gross A et al. 2001; Kim et al. 2010), but there is no report showing that CNIs suppress TDAR through the changes in IL-2R expression in these cells.

Empirical Evidence
The relationship of the interference of nuclear localization of NFAT leading to reduced NFAT/AP-1 complex formation bound at the promoter sites of cytokine genes in the presence of CNIs is well known as mentioned above.

Imaging flowcytometry revealed that concentration-dependent reduction of in vitro nuclear localization of NFAT was evident at the maximum concentration of 1 μM with minimal concentration of 0.1nM (Jurkat human T cell line) or 10nM ($CD4^+$T cells from whole blood) after 2 hours treatment of tacrolimus (Maguire et al. 2013).

The experiment of gel mobility shift assay using Ar-5 human T cells stimulated with cross-linked anti-CD3 antibody showed that NFAT/AP-1 (cFos and Jun) complexes were found only in the nuclear extract with preexisting NFAT in the cytoplasm after T cell stimulation and that the NFAT/AP-1 complexes in the nucleus decreased after 2 hours treatment with CsA at 1μM (Jain et al. 1992). Decreased NFAT translocated to the nucleus, induced by FK506 at 100ng/mL (0.125nM) or CsA at 500ng/mL (0.416nM) for 2 hours treatment, hinders the formation of the functional NFAT/AP-1 complexes necessary to binding at the site of IL-2 promoters (Flanagan et al. 1991)NFAT/AP-1 complex formation is inhibited by CNI (Rao et al. 1997).

Quantitative understanding of NFAT/AP-1 complex formation in the nucleus is insufficient although nuclear localization of NFAT and following nuclear NFAT/AP-1 complex formation are shown to be tightly related; therefore, NFAT nuclear translocation and complex formation with AP-1 in in the nucleus deem to be suppressed by CNIs under the same conditions of dose ranges and treatment duration..

Uncertainties and Inconsistencies
Nothing especially

Quantitative Understanding of the Linkage

Response-response relationship
The relationship of the interference of nuclear localization of NFAT leading to reduced NFAT/AP-1 complex formation bound at the promoter sites of cytokine genes in the presence of CNIs is well known as mentioned above.

KE1:

Dose-dependent interference with nuclear translocation of NFAT1 was observed with increasing FK506 concentrations from 0.01nM (Jarkat T cells) up to 1 μM (1000 nM). Higher concentrations induced cellular toxicity and resulted in cell death. Dose-dependent interference of nuclear NFAT1 translocation per CN inhibition was also observed in CD4+ T cells from healthy donors, again from 10nM to maximal concentrations of 1 μM (Maguire et al. 2013). Both parameters were measured after 2 hour culture of T cells with FK506.

KE2:

Reduction in generation of NFAT/AP-1 complexes can be detected using a gel shift assay (Rao et al. 1997, Jain et al. 1992, Jain et al. 1993).

Decreased NFAT translocated to the nucleus, induced by FK506 at 100ng/mL (124nM) or CsA at 500ng/mL (416nM) after 2 hours treatment, hinders the formation of the functional NFAT/AP-1 complexes necessary to binding at the site of IL-2 promoters (Flanagan et al. 1991). As mentioned above, the gel mobility shift assay also showed that NFAT/AP-1 complexes were formed only in the nucleus after T cell activation with unchanged preexisting NFAT in the cytoplasm and that treatment of T cells with 1μM FK506 led to decease the levels of NFAT/AP-1 complex (Jain et al. 1992).

These findings suggest that nuclear translocation of NFAT after T cell stimulation is strongly related to the complex formation with AP-1 in the nucleus, and FK506 was shown to inhibit NFAT/AP-1 complex formation in the nucleus at the concentrations within the concentration range of FK506 for suppressing nuclear translocation of NFAT (Maguire et al. 2013).

Time-scale
Nuclear translocation of NFAT was shown to be inhibited in vitro using imaging flowcytometry after 2 hours culture of T cells with FK506 (Maguire et al. 2013), and gel mobility shift assay revealed the inhibition of nuclear translocation of NFAT and following complex formation with AP-1 within the nucleus after 2 hours culture of T cells with FK506 (Jain et al. 1992, Flanagan et al. 1991).

Known modulating factors
(To be described)

Known Feedforward/Feedback loops influencing this KER
(To be described)

References

1. Bhattacharyya, S., Deb, J., Patra, A.K., Thuy Pham, D.A., Chen, W., Vaeth, M., Berberich-Siebelt, F., Klein-Hessling, S., Lamperti, E.D., Reifenberg, K., Jellusova, J., Schweizer, A., Nitschke, L., Leich, E., Rosenwald, A., Brunner, C., Engelmann, S., Bommhardt, U., Avots, A., Müller, M.R., Kondo, E. and Serfling, E. (2011). NFATc1 affects mouse splenic B cell function by controlling the calcineurin-NFAT signaling network. The Journal of experimental medicine 208 (4): 823-39.
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.
3. Jain, J., McCaffrey, P. G., Valge-Archer, V. E. and Rao, A. (1992). Nuclear factor of activated T cells contains Fos and Jun. Nature. 356(6372): 801-4.
4. Jain, J., Miner, Z. and Rao, A. (1993). Analysis of the preexisting and nuclear forms of nuclear factor of activated T cells. Journal of immunology. 151(2): 837-48.
5. Kim, T., Kim, N. and Kang, H. J. (2010). FK506 causes cellular and functional defects in human natural killer cells. Journal of leukocyte biology. 88:1089-1097.
6. Maguire O, Tornatore KM, O'Loughlin KL, Venuto RC and Minderman H. (2013) Nuclear translocation of nuclear factor of activated T cells (NFAT) as a quantitative pharmacodynamic parameter for tacrolimus. Cytometry A. 83(12):1096-104.
7. Matsuda, S., Koyasu, S. (2000). A second target of cyclosporin A and FK506. Tanpakushitsu kakusan koso. 45(11): 1823-31.
8. Panhans-Gross, A., Novak, N., Kraft, S., and Bieber, T. (2001). Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). Journal of Allergy and Clinical Immunology 107(2): 345-52.
9. Rao, A., Luo, C., and Hogan, PG. (1997). Transcription factors of the NFAT family: regulation and function. Annual Review of Immunology 15: 707-47.
10. Schreiber, SL., and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42.
11. Zhu, J. and McKeon, F. (1999). NF-AT activation requires suppression of Crm1-dependent export by calcineurin. Nature. 398(6724): 256-60.

Relationship: 1509: Reduction, NFAT/AP-1 complex formation leads to Suppression, IL-2 and IL-4 production (https://aopwiki.org/relationships/1509)

AOPs Referencing Relationship

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

In purified T cell from male C57BL/6J mice, T-5224 (a selective c-Fos/AP-1 inhibitor) inhibits the DNA-binding activity of AP-1, IL-2 production and CD25 (IL-2 receptor) up-regulation (Yoshida et al. 2015).

In splenic lymphocytes and/or CD4+ T cells, ursolic acid suppressed products of NF-kB, NFAT and AP-1, and inhibits secretion of IL-2 and IL-4, mRNA level of IL-2 and CD25 expression (Checker et al. 2012).

NFATp- and NFAT4-deficient mice indicate decreased production of IL-2 (Ranger et al. 1998).

NFAT/AP-1 complex formation in the nucleus was shown using murine and human T cells lines (Jain J et al. 1992). In addition to data on suppression of cytokine production by CNI in rodents, the following is known from the use of human cells: FK506 inhibited expression of both IL-2 and mRNA in human anti-CD3/PMA-activated cells (Dumont et al. 1998).

Key Event Relationship Description

Localized NFAT in the nucleus of T cells binds to form complexes with AP-1 at the IL-2 promoter region (Schreiber and Crabtree 1992; Jain et al. 1992), which induces transcription of IL-2 (Jain et al. 1993). 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.

For IL-2, NFAT proteins are necessary for IL-2 gene expression and cooperation of NFAT with AP-1 is required for IL-2 gene transcription. For IL-4, At least five different NFAT sites have been described in the IL-4 promoter with at least three of them being composite sites binding NFAT and AP-1 (Macián et al. 2001).

Decreased formation of NFAT/AP-1 complex at the promoter region of IL-2 genes in the nucleus of T cells following lowed nuclear localization of NFAT by CNI treatment reduces the transcription of IL-2 (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 (Dumont et al. 1998).

Evidence Supporting this KER

Biological Plausibility
T-5224, a selective c-Fos/AP-1 inhibitor, inhibits the DNA-binding activity of AP-1 in primary murine T cells. T-5224 also inhibits CD25 (one of IL-2 receptors) up-regulation, IL-2 production, and c-Fos DNA-binding activity in mice (Yoshida et al. 2015).

Dexamethasone represses the IL-2 mRNA induction. GILZ (glucocorticoid-induced leucine zipper) is one of the most prominent glucocorticoid-induced genes, and inhibited the induction of the NFAT reporter and interferes with the AP-1 component of the NFAT/AP-1 complex. GILZ also inhibits the IL-2 promoter (Mittelstadt et al. 2001).

Ursolic acid suppressed activation of three immunoregulatory transcription factors NF-kB, NFAT and AP-1. Treatment of lymphocytes and CD4+ T cells with ursolic acid inhibited secretion of IL-2 and IL-4 cytokines. Treatment of CD4+ T cells with ursolic acid suppressed mRNA level of IL-2. Treatment of lymphocytes with ursolic acid inhibited the upregulation of CD25 expression on T cells (Checker et al. 2012).

NFATp- and NFAT4-deficient mice indicate decreased production of Th1 cytokine including IL-2 (Ranger et al. 1998).

It is generally accepted that NFAT, translocated to the nucleus after T-cell stimulation, binds with AP-1 to the promoter regions of the cytokine genes to mount transcription, which follows production of these T-cell–derived cytokines. Of these cytokines, IL-2 and IL-4 promote proliferation, maturation, and class-switching of B cells to enhance TDAR.

There is also sufficient evidence to support the hypothesis that CNI-induced decreases in T-cell–derived cytokine production is mediated through suppressed nuclear localization of NFAT, with a resultant decrease in the amount of NFAT/AP-1 complex binding to the promoter regions of T-cell–derived cytokines.

When stimulated with ovalbumin, CnA-KO mice produce less IFN-g, IL-2, and IL-4 than wild-type mice. However, primary antibody response in CnA-KO mice is normal in response to TNP-ovalbumin (Zhang et al. 1996).

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).

Therefore, the study of NFAT-KO mice shows that NFAT is involved in a wide range of immune responses, and some of these phenomenon are known to be regulated by CN. Suppression of T-cell-derived cytokines is noted both in CnA-knockout and NFAT-knockout mice, which indicates that the production of T-cell derived cytokines such as IL-2 and IL-4 is regulated by the CN-NFAT system.

FK506-FKBP12 complex decreased CN phosphatase activity, which leads to inhibit translocation of NFAT to the nucleus. Because NF-ATp is an essential transcription factor regulating the IL-2 gene, FK506 ultimately blocks the T-cell response by inhibiting IL-2 transcription (Panhans-Gross A et al. 2001). FK506 inhibited IL-2 mRNA expression in anti-CD3/PMA-activated cells (Dumont et al. 1998).

These facts indicate that although NFAT is widely involved in the function of T cells, the effect of CNIs is to suppress production of some classes of T-cell–derived cytokines through reducing the formation of NFAT/AP-1 complexes induced by inhibition of CN phosphatase activity.

Empirical Evidence
Empirical support of Reduction, NFAT/AP-1 complex formation leading to Suppression, IL-2 and IL-4 production is strong.

Rationale

- In purified T cell from male C57BL/6J mice, T-5224 (a selective c-Fos/AP-1 inhibitor) inhibits the DNA-binding activity of AP-1 and CD25 (one of IL-2 receptors) up-regulation at 80 μg/mL, and IL-2 production in a dose-dependent manner from 40 to 80 μg/mL (Yoshida et al. 2015).
- In splenic lymphocytes stimulated with concanavalin A for 24 h in C57BL/6 mice, ursolic acid suppressed products of NF-kB, NFAT and AP-1 at 5 μM for 4 h. Secretion of IL-2 and IL-4 was inhibited in lymphocytes stimulated with concanavalin A for 24 h at concentrations of 0.5, 1 and 5 μM of ursolic acid, and lymphocytes and CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb for 24 h at concentration of 5 μM of ursolic acid. In CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb for 24 h, ursolic acid suppressed mRNA level of IL-2 at 5 μM for 4 h. In lymphocytes stimulated with concanavalin A for 24 h, ursolic acid inhibited CD25 expression at 5 μM for 4 h (Checker et al. 2012).
- In NFATp- and NFAT4-deficient mice, cultured splenocytes bound anti-CD3 for 48 h indicates decreased production of Th1 cytokine including IL-2 (Ranger et al. 1998).
- It is well established that inhibition of NFAT/AP-1 complex formation at the promoter sites reduces the production of T-cell–derived cytokines including IL-2 and IL-4, which are mainly involved in T-cell–dependent antibody response.
- NFAT/AP-1 complex formation is inhibited by CNI shown by gel shit mobility assay using human T cell line or CD4+ T cells from heathy donners after 2 hours treatment with CsA at 1μM.. Preceding NFAT nuclear localization after T cell activation is suppressed with FK506 at the dose range of 0.01nM (Jarkat T cells) or 10nM (CD4+ T cells) to 1μM (Maguire et al. 2013), and NFAT nuclear localization and NFAT/AP-1 complex formation is shown to be strongly related (Jain et al. 1992, Jain et al. 1993).
- In CD3/PMA-activated human T cells, FK506 suppressed production of IL-2, IL-4, and IFN-γ at the concentrations of 1.2 to 12.5 nM after 22 to 24 hours culture as well as inhibited expression of IL-2, IL-4, and IFN-γ mRNA in a dose-dependent (10 nM) manner after 3 day culture (Dumont et al. 1998).

Reduced nuclear translocation of NFAT followed by NFAT/AP-1 complex formation and suppression of IL-2/IL-4 productions are shown to occur under similar dose ranges and treatment duration.

Uncertainties and Inconsistencies
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 these FK506-induced mechanisms and NFAT and contribution of those to TDAR are unclear.

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; however, contribution of these additional transcription factors to TDAR is also unclear.

Quantitative Understanding of the Linkage

Response-response relationship
In purified T cells from male C57BL/6J mice, T-5224 (a selective c-Fos/AP-1 inhibitor) inhibits the DNA-binding activity of AP-1 at 80 μg/mL. On the other hand, T-5224 inhibits IL-2 production in a dose-dependent manner from 40, 60 and 80 μg/mL after 48 hours culture. T-5224 also inhibits CD25 (IL-2 receptor) up-regulation at 80 μg/mL (Yoshida et al. 2015).

In splenic lymphocytes stimulated with concanavalin A for 24 h in C57BL/6 mice, ursolic acid suppressed products of NF-kB, NFAT and AP-1 at 5 μM. In lymphocytes stimulated with concanavalin A for 24 h, ursolic acid inhibits secretion of IL-2 and IL-4 at 0.5, 1 and 5 μM. In lymphocytes and CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb for 24 h, ursolic acid also inhibits secretion of IL-2 and IL-4 at 5 μM. In CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb for 24 h, ursolic acid suppressed mRNA level of IL-2 at 5 μM. In lymphocytes stimulated with concanavalin A for 24 h, ursolic acid inhibited CD25 expression at 5 μM (Checker et al. 2012).

These findings showed that T-5244 and ursolic acid treated for 24 hours inhibit NFAT/AP-1 complex formation at a single concentration each and that these compounds suppress IL-2 and IL-4 production with dose dependent manner including the doses for inhibition of NFAT/AP-1 complex formation.

FK506 suppressed proliferation in human T cells induced by anti-CD3 mAb in the presence of adherent autologous PBMC (mean IC50 = 0.06 nM). FK506 suppressed, in a dose-dependent (1.2 to 12.5 nM) manner after 22-24 hours culture, 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). On the other hand, the quantitative data for the decreased formation of NFAT/AP-1 complexes by CNI is insufficient, although the formation was suppressed by FK506 at the concentration within the range needed for suppressed production of IL2/IL-4 by FK506 after 2 hours culture.

Time-scale
Inhibition of NFAT/AP-1 complex is detected by gel mobility shit assay after 2 hours culture with CNI; however, suppression of IL2/IL-4 could be measured after 22-48 hours in vitro culture.

Known modulating factors
(To be described)

Known Feedforward/Feedback loops influencing this KER
(To be described)

References

1. Schreiber, SL., and Crabtree, GR. (1992). The mechanism of action of cyclosporin A and FK506. Immunology Today 13(4): 136-42.
2. Jain J., McCaffrey P.G., Valge-Archer V.E., Roa A.(1992). Nuclear factor of activated T cells contains Fos and Jun. Nature 356(6372):801-804.
3. Jain J., Miner Z., Rao A. (1993). Analysis of the preexisting and nuclear forms of nuclear factor of activated T cells. Journal of Immunology 151(2): 837-848.
4. Macián, F., López-Rodríguez, C. and Rao, A. (2001). Partners in transcription: NFAT and AP-1. Oncogene. 20(19): 2476-89.
5. Yoshida, T., Yamashita, K., Watanabe, M., Koshizuka, Y., Kuraya, D., Ogura, M., Asahi, Y., Ono, H., Emoto, S., Mizukami, T., Kobayashi, N., Shibasaki, S., Tomaru, U., Kamachi, H., Matsushita, M., Shiozawa, S., Hirono, S. and Todo, S. (2015). The Impact of c-Fos/Activator Protein-1 Inhibition on Allogeneic Pancreatic Islet Transplantation. Am J Transplant. 15(10): 2565-75.
6. Mittelstadt, PR. and Ashwell, JD. (2001). Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ. J Biol Chem. 276(31):29603-10.
7. Checker, R., Sandur, SK., Sharma, D., Patwardhan, RS., Jayakumar, S., Kohli, V., Sethi, G., Aggarwal, BB. and Sainis, KB. (2012). Potent Anti-Inflammatory Activity of Ursolic Acid, a Triterpenoid Antioxidant, Is Mediated through Suppression of NF-κB, AP-1 and NF-AT PLoS One. 7(2): e31318.
8. Ranger, AM., Oukka, M., Rengarajan, J. and Glimcher, LH. (1998). Inhibitory function of two NFAT family members in lymphoid homeostasis and Th2 development. Immunity. 9(5):627-35.
9. Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.
10. Macian, F. (2005) NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol. 5(6): 472-84.
11. Panhans-Gross, A., Novak, N., Kraft, S., and Bieber, T. (2001). Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). Journal of Allergy and Clinical Immunology 107(2): 345-52.
12. van Dieren, J.M., Lambers, M.E.H., Kuipers, E.J., Samsom, J.N., van der Woude, C.J. and Nieuwenhuis, E.E.S. (2010). Local immune regulation of mucosal inflammation by tacrolimus. Digestive diseases and sciences 55(9): 2514-19.
13. Zhang, BW., Zimmer, G., Chen, J., Ladd, D., Li, E., Alt, FW., Wiederrecht, G., Cryan, J., O'Neill, EA., Seidman, CE., Abbas, AK., Seidman, JG. (1996). T cell responses in calcineurin A alpha-deficient mice. J Exp Med. 183(2): 413-20.

Relationship: 1510: 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	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Calcineurin Activity Leading to Impaired T-Cell Dependent Antibody Response (https://aopwiki.org/aops/154)	**adjacent**	**High**	**High**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Suppressed IgE and antigen specific IgG1 productions by the blocking of IL-4 receptor were reported in mice using dupilumab (anti-IL-4/13 receptor antibody) (Sanofi K.K. 2018).

Suppressed antigen specific IgE production by the inhibition of IL-4 production was reported in mice using suplatast tosilate (an inhibitor of the production of cytokines on Th2 cell) (Taiho Pharmaceutical 2013).

Suppressed antigen specific IgE and IL-4 productions by the inhibition of IL-4 production were reported in human cell culture using suplatast tosilate(Taiho Pharmaceutical 2013).

The effects of FK506 on serum concentration of anti-KLH antibodies IgM and IgG have been demonstrated in rats treated with FK506 for over four weeks and immunized with KLH (Ulrich et al. 2004). The effects of FK506 and CsA on antigen-specific plaque-forming splenocytes have been demonstrated in mice treated with FK506 or CsA for 4 days and immunized with SRBC (Kino et al. 1987b).

The effects of FK506 and CsA on the levels of IgM and IgG in the culture supernatant have been demonstrated in human cells (Heidt et al, 2009, Sakuma et al, 2001).

The effects of FK506 and CsA on production of IL-2 and IL-4 have been demonstrated using mice and human cells (Kino et al. 1987a, Dumont et al. 1998).

These facts suggest that there are no species differences between humans and rodents in inhibitions of IL-2 and IL-4 production and TDAR induction.

Key Event Relationship Description

IL-2 and IL-4 are produced and secreted by helper T cells and play important roles in the development of TDAR. IL-4 affects maturation and class switching of B cells as well as proliferation, both of which induces/enhances T cell dependent antibody production. IL-2 promotes differentiation of B cells through IL-2 stimulates differentiation of the activated T cell into T cell called Th2 cell. Therefore, suppressed production of IL-2 and IL-4 impairs T cell dependent antibody production (Alberts et al. 2008).

T cells, B cells, and antigen-presenting cells such as dendritic cells are involved in inducing and developing of TDAR. Thus, changes in any of these immune cell populations can influence TDAR

T cell-derived cytokines play important roles in the development of TDAR. Among them, IL-2 promotes proliferation of B cells, and IL-4 affects maturation and class switching of B cells as well as proliferation, both of which induces/enhances T cell dependent antibody production.

Thus, after treatment with FK506, production of IL-2, IL-4, and other cytokines decreases in T cells (Dumont et al. 1998)., reducing stimulation of B cells as well as proliferation, activation, and class switching, and leading to impairment of TDAR. Therefore, FK506 and cyclosporin A (CsA) are potent inhibitors of T-cell–dependent-antibody production and suppressing the production of these B-cell-related cytokines appears to be the main factor in impairment of TDAR by FK506 (Heidt, S. et al. 2009).

Evidence Supporting this KER

Biological Plausibility
Dupilumab is known as anti-IL-4/13 receptor antibody. Dupilumab (Dupixent) reduces productions of IgE and antigen specific IgG1 in mice (Sanofi K.K. 2018). It suggests that the blocking of IL-4 signaling by anti-IL-4/13R antibody results in the decrease in T cell dependent antibody production.

Th2 cell produces cytokines including IL-4. Suplatast tosilate (IPD) is known as an inhibitor of the production of cytokines on Th2 cell. Suplatast tosilate reduces the production of antigen specific IgE in human cell culture and mice (Taiho Pharmaceutical 2013). It suggests that the reduction of IL-4 production by the inhibitor of the production of cytokines on Th2 cell results in reduced production of IgE through inhibitions of maturation, proliferation and class switching of B cell.

IL-2 binds to IL-2 receptor (IL-2R) and acts on T cell. CD25 is a one of IL-2R. Basiliximab (Simulect) is known as anti-CD25 antibody. Basiliximab binds to IL-2R and blocks IL-2 signaling. Clinical transplantation study of basiliximab reveals decreases in rejections. On the other hand, basiliximab inhibits the activation of antigen specific T cell (Novartis Pharma 2016). They suggest that the blocking of IL-2 signaling by anti-IL-2R antibody results in decreased rejection through the inhibition of the activation of antigen specific T cell with reduced antibody production.

FK506 and CsA suppress mRNA expression levels of cytokines in T cells including IL-2 and IL-4 that stimulate proliferation of B cells as well as B cell activation and class switching (Heidt et al, 2009). It is established that IL-2 stimulates B cells to proliferate through the surface IL-2 receptors and that IL-4 stimulates B cells to proliferate, to induce class switch, and to differentiate into plasma and memory cells.

Several in vivo studies in rodents showed decreased TDAR by the treatment of FK506 (Kino et al. 1987b, Ulrich et al. 2004). In in vitro tests examining antibody production in blood samples obtained from blood-bank donors, PBMC treated with FK506 and CsA suppressed the production of immunoglobulin (Ig) M and G antibodies to T-cell dependent antigens (Heidt et al, 2009).

T cells, B cells, and antigen-presenting cells such as dendritic cells are involved in inducing and developing of TDAR. Thus, changes in any of these immune cell populations can influence TDAR.

However, as for the suppression of humoral immunity induced by the inhibition of CN phosphatase activity,, calcineurin inhibitors (CNIs) do not affect B cells directly but rather indirectly through T cells. That is, FK506 and CsA are capable of inhibiting immunoglobulin production when B cells are cultured with non-pre-activated T cells, but FK506 and CsA fail to inhibit immunoglobulin levels when pre-activated T cells are used to stimulate B cells. Hence, the inhibition of B cell response by FK506 and CsA appears due solely to inhibition of T helper cells (Heidt et al, 2009).

Therefore, it is concluded that decreased amounts of IL-2 and IL-4 secreted from helper T cells is the main factor for suppression of TDAR induced by CN phosphatase inhibition.

Empirical Evidence
Empirical support of the suppression, IL-2 and IL-4 production leads to impairment, T-cell dependent antibody response is strong.

Rationale

- In the allergen-induced pneumonia model in mice, dupilumab (anti-IL-4/13 receptor antibody) reduced productions of IgE and antigen specific

IgG1 at 25 mg/kg of twice weekly subcutaneous administration for 4weeks (Sanofi K.K. 2018).

- In mice immunized with dinitrophenyl antigen by i.p. injection, suplatast tosilate (an inhibitor of the production of cytokines on Th2 cell) reduced productions of antigen specific IgE at 10, 20, 50 and 100 mg/kg of oral administration for 5 days (Taiho Pharmaceutical 2013). In human cell culture immunized with Japanese cedar antigen, suplatast tosilate reduced productions of antigen specific IgE at the concentration of 10 μg/mL for 10 days (Taiho Pharmaceutical 2013).
- In the clinical study of renal transplantation, basiliximab decreased incidence of acute rejection at 20 mg/kg (Novartis Pharma 2016). In human T cell culture immunized with PPD, basiliximab reduced activation of antigen specific T cell at the concentration of 300 ng/mL (Novartis Pharma 2016).
- In CD3/PMA-activated human T cells, FK506 suppressed production of IL-2, IL-4 and Interferon (IFN)-γ at the concentrations of 1.2 to 12.5 nM as well as inhibited expression of IL-2, IL-4 and IFN-γ mRNA at the concentrations of 10 nM. (Dumont et al. 1998).
- FK506 or CsA suppressed production of IL-2 in mouse mixed lymphocyte reaction (MLR) at 0.1 to 10 nM of FK506 and 10 to 100 nM of CsA as well as in human MLR at 0.1 to 10 nM of FK506 and 10 to 100 nM of CsA (Kino et al. 1987a).
- After 9-day culture of B cells and non-pre-activated T cell stimulationwith FK506 or CsA, the levels of IgM and IgG in the culture supernatant were reduced at 0.3 and 1.0 ng/mL (0.37 and 1.24 nM) of FK506 or 50 and 100 ng/mL (41 and 83nM) of CsA (Heidt et al, 2009).
- After 4-day culture of SKW6.4 cells (IL-6-dependent IgM-secreting human B-cell line) and anti-CD3/CD28 stimulated PBMC culture supernatant with FK506 or CsA, the level of IgM in the culture supernatant was reduced at the concentrations of 0.01 to 100 ng/mL (0.01 to 124 nM) of FK506 or 0.1 to 1000 ng/mL (0.08 to 832 nM) of CsA (Sakuma et al, 2001).
- Rats were treated with FK506 for over four weeks and immunized with KLH, after which serum concentration of anti-KLH IgM and IgG reduced at the dose levels of 3 mg/kg/day (Ulrich et al. 2004).
- Mice were treated with FK506 or CsA for 4 days, and immunized with SRBC, after which antigen-specific plaque-forming splenocytes reduced at the dose levels of 3.2, 10, 32 and 100 mg/kg of FK506 or 32 and 100 mg/kg of CsA (Kino et al. 1987b).

In vitro suppression of T-cell–derived cytokines and T-cell-dependent antibody production or antibody production after polyclonal T-cell stimulation showed similar dose responses to CNIs. Time gaps were found, however, between these two KEs, which showed earlier onset of cytokine production and delayed onset of antibody production.

Uncertainties and Inconsistencies

IL-2 affects multiple populations of immune cells expressing IL-2 receptors, while IL-4 mainly acts on B cells. Therefore, reduced production of both IL-2 and IL-4 might certainly induce suppression of TDAR; however, there remains some possibility of additional suppression of other immune functions.

Quantitative Understanding of the Linkage

Response-response relationship

In the blocking of IL-4 receptor in mice by dupilumab (anti-IL-4/13 receptor antibody) at 25 mg/kg of twice weekly subcutaneous administration for 4weeks, IgE production was suppressed to about 1/100 and antigen specific IgG1 production was suppressed to about 1/200 (Sanofi K.K. 2018).

In the inhibition of IL-4 production in mice by suplatast tosilate (an inhibitor of the production of cytokines on Th2 cell) at 10, 20, 50 and 100 mg/kg of oral administration for 5 days, antigen specific IgE production was suppressed from about 1/10 to 1/100 (Taiho Pharmaceutical 2013). In human T cell culture by suplatast tosilate at the concentration of 10 μg/mL, antigen specific IgE production after 10 days was suppressed from 56 to 72% and IL-4 production after 3 days was suppressed from 58 to 76% (Taiho Pharmaceutical 2013).

As for IL-2 and antibody production, in vitro T-cell-induced polyclonal B cell activation to produce antibody was inhibited with anti-IL-2 and anti-IL-2R antibodies. That is, murine small resting B cells, cultured with irradiated hapten-specific TH1 clone, were induced to enter cell cycle at 2 days and to secret antibody at 5 days. An anti-IL-2 and anti-IL-2R antibodies completely inhibited this T-cell dependent antibody production (Owens T, 1991).

In addition, cynomolgus monkeys treated wth CsA showed suppression of IL-2 and TDAR using sheep red blood cells with a dose dependent manner (Gaidal K. 2015).

In the human T-B cell co-culture stimulated with anti-CD3 monoclonal antibody, CNIs of FK506 and CsA lowered the m-RNA levels of T-cell cytokines at 8h post-stimulation including IL-2 and IL-4 at 1.0ng/mL (1.24nM) FK506 or 100ng/mL (90.7nM) CsA and inhibited IgM and IgG productions after 9 days at 0.3 and 1.0ng/mL FK506 and 50 and 100ng/mL CsA (Heidt S. 2010).

Time-scale

In human T cell culture, suplatast tosilate (an inhibitor of the production of cytokines on Th2 cell) inhibits IL-4 production after 3 days and antigen specific IgE production after 10 days (Taiho Pharmaceutical 2013).

In the human T-B cell co-culture, CNIs of FK506 and CsA lowered the m-RNA levels of IL-2 and IL-4 at 8h post-stimulation and inhibited IgM and IgG productions after 9 days (Heidt S. 2010).

Known modulating factors

(To be described)

Known Feedforward/Feedback loops influencing this KER

(To be described)

References

1. Alberts, B., Johnson, A., Lewis, L., Raff, M., Roberts, K. and Walter, P. (2008). Molecular Biology of the Cell. 5th ed., Garland Science, New York. 1539-1601
2. Sanofi K.K. (2018) Drug interview form Dupixent subcutaneous injection 300 mg syringe. 2nd edition.
3. Taiho Pharmaceutical Co.,Ltd. (2013) Drug interview form IPD capsule 50 and 100. Revised 5th edition.
4. Novartis Pharma K.K. (2016). Drug interview form Simulect i.v. injection 20 mg. 10th edition.
5. Dumont, F.J., Staruch, M.J., Fischer, P., DaSilva, C. and Camacho, R. (1998). Inhibition of T cell activation by pharmacologic disruption of

the MEK1/ERK MAP kinase or calcineurin signaling pathways results in differential modulation of cytokine production. Journal of immunology 160 (6): 2579-89.

6. Heidt, S., Roelen, D. L., Eijsink, C., Eikmans, M., van Kooten, C., Claas, F. H. and Mulder, A. (2010). Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. Clinical and experimental immunology. 159(2): 199-207.
7. Gaida K., Salimi-Moosavi H., Subramanian R., Almon V., Knize A., Zhang M., Lin F.F., Nguyen H.Q., Zhou L., Sullivan J.K., Wong M., McBride H.J. (2015). Inhibition of CRAC with a human anti-ORAI1 monoclonal antibody inhibits T-cell-derived cytokine production but fails to inhibit a T-cell-dependent antibody response in the cynomolgus monkey. J Immunotoxicol 12:164-173.
8. Heidt S., Roelen D.L., Eijsink C., Eikmans M., van Kooten C., Claas F.H., Mulder A.(2010). Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help.
9. Kino, T., Hatanaka, H., Miyata, S., Inamura, N., Nishiyama, M., Yajima, T., Goto, T., Okuhara, M., Kohsaka, M. and Aoki, H. (1987a). FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. Journal of antibiotics. 40(9): 1256-1265.
10. Kino, T., Hatanaka, H., Hashimoto, M., Nishiyama, M., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Imanaka, H. (1987b). FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. Journal of antibiotics. 40(9): 1249-1255.
11. Owens T.(1991). Requirement for noncognate interaction with T cells for the activation of B cell immunoglobulin secretion by IL-2. Cell Immunol 133:352-366.
12. Sakuma, S., Kato, Y., Nishigaki, F., Magari, K., Miyata, S., Ohkubo, Y., and Goto, T. (2001b). Effects of FK506 and other immunosuppressive anti-rheumatic agents on T cell activation mediated IL-6 and IgM production in vitro. International Immunopharmacology 1(4): 749-57.
13. Ulrich, P., Paul, G., Perentes, E., Mahl, A., and Roman D. (2004). Validation of immune function testing during a 4-week oral toxicity study with FK506. Toxicology Letters 149(1-3): 123-31.