UNIFIED MODELING OF FAMILIAL MEDITERRANEAN FEVER AND CRYOPYRIN ASSOCIATED PERIODIC SYNDROMES

by

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This is to certify that I have examined this copy of a master's thesis by

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To my family

Nurettin, Kübra and Alper Bozkurt

ABSTRACT

Familial Mediterranean Fever (FMF) and Cryopyrin Associated Periodic Syndromes (CAPS) are two related autoinflammatory diseases, characterized by recurrent episodes of fever and inflammation. FMF is caused by inherited loss-of-function mutations in pyrin, and CAPS by gain-of-function mutations in cryopyrin, two proteins that play key roles in the control and regulation of inflammation along with an enzyme, Caspase 1, and a cytokine, IL-1β. Normally, triggered by an infection or a sterile insult, cryopyrin forms an inflammasome that converts precursor proCaspase 1 into active Caspase 1. Once activated, Caspase 1 proteolytically cleaves IL-1β into an active peptide with a proinflammatory effect. Pyrin, on the other hand, acts as an anti-inflammatory mediator that controls and prevents cryopyrin inflammasome formation by binding both to Caspase 1 and its inactive form proCaspase 1. Mutated pyrin can no longer act as an effective suppressor of inflammasome formation. Mutations in cryopyrin, on the other hand, result in elevated inflammasome formation even in the absence of a trigger. Overproduction of IL-1β by Caspase 1 as a result is the main cause of fever and inflammatory episodes in FMF and CAPS.

In this thesis, we present a unifying dynamical model for FMF and CAPS in the form of coupled nonlinear ordinary differential equations. The model is composed of two subsystems. The first subsystem captures the interactions and dynamics of pyrin, cryopyrin, proCaspase 1, Caspase 1, and inflammasome formation, including the effect of triggers. The second subsytem, which contains a coupled positive-negative feedback motif, captures the dynamics of IL-1β, its receptor and receptor antagonist, including Caspase 1-independent cleavage of IL-1β. The two subsystems are coupled via the key player Caspase 1. We perform a comprehensive bifurcation analysis of the model and show that it exhibits three modes, capturing the *Healthy*, *FMF* and *CAPS* cases. The mutations in pyrin and cryopyrin are reflected in the values of three parameters in the model. We then present extensive simulation results for the model, which match clinical observations. In the presence of

a trigger, there is a normal increase in inflammation initiators in the *Healthy* mode. In *FMF*, the response to trigger introduction is more intense and a severe inflammatory cascade is activated. In *CAPS*, on the other hand, even in the absence of a trigger, periodically recurring, severe inflammatory episodes are observed. The proposed model also explains why a proCaspase 1 inhibitor can be effective in treating FMF, but not CAPS, for which drugs that directly inhibit IL-1β are used.

ÖZETÇE

Ailevi Akdeniz Ateşi (FMF) ve Kriyopyrin İlişkili Periyodik Sendromlar (CAPS) ilişkili iki otoinflamatuar hastalıktır ve ateş ve inflamasyon atakları ile karakterizedir. FMF, pyrin proteindeki fonksiyon kaybedici, CAPS ise kriyopyrin proteinindeki fonksiyon kazandırıcı mutasyonlar sonucu ortaya çıkar. Bu iki protein, Kaspaz 1 enzimi ve interlökin-1β (IL-1β) sitokini ile birlikte inflamasyon kontrolü ve düzenlemesinde kritik rol oynar. Normal olarak, bir enfeksiyon ya da steril inflamasyon tarafından tetiklendiğinde, kriyopyrin, proKaspaz 1'i aktif Kaspaz1'e dönüştüren bir inflamazom oluşturur. Aktif hale gelen Kaspaz 1, proteolitik kesme yoluyla IL-1β'yı proinflamatuar etkisi olan aktif haline çevirir. Öte yandan, pyrin, Kaspaz1'e ve onun inaktif formu olan proKaspaz1'e bağlanma yoluyla kriyopyrin inflamazom oluşumunu kontrol eden ve engelleyen bir anti-inflamatuar mediatör olarak davranır. Mutasyona uğramış pyrin inflamazom formasyonunu baskılayıcı rolünü etkili bir biçimde gerçekleştiremez. Diğer taraftan, kriyopyrindeki mutasyon bir tetikleyici yokluğunda bile inflamazom formasyonunda artışla sonuçlanır. Sonuç olarak, IL-1β aşırı üretimi FMF ve CAPS ateş ve inflamasyon ataklarının temel sebebidir.

Bu tezde, FMF ve CAPS için eşleşmiş lineer olmayan adi diferansiyel denklem formunda bir bütünleşik model sunuyoruz. Bu model iki alt sistemden oluşmaktadır. İlk alt sistem, pyrin, kriyopyrin, proKaspaz 1, Kaspaz 1 ve inflamazom formasyonundaki etkileşim ve dinamikleri, tetikleyici etkisi de dahil edilecek şekilde içerir. Pozitif ve negatif geri besleme motiflerini kapsayan ikinci alt sistem, IL-1β, IL-1β reseptörü ve antagonisti ve Kaspaz 1'a bağlı olmayan IL-1β kesimi dinamiklerini içerir. Bu iki alt sistem Kaspaz 1 önemli bileşeni yoluyla eşleşmiştir. Modelin kapsamlı bir çatallanma analizi yapılmıştır ve *Sağlıklı*, *FMF* ve *CAPS* olmak üzere üç mod sergilediği gösterilmiştir. Pyrin ve kriyopyrindeki mutasyonlar, modeldeki üç parametrenin değeri ile yansıtılmıştır. Ayrıca, modelin klinik gözlemlerle örtüşen kapsamlı simülasyon sonuçları gösterilmiştir. *Sağlıklı* modda, tetikleyici varlığında inflamasyon başlatıcılarında normal bir artış görülür.

FMF modunda, tetikleyici girişlerine tepki daha yoğundur ve şiddetli bir şekilde inflamasyon kaskadı aktif hale gelir. Öte yandan *CAPS* modunda, tetikleyici yokluğunda dahi periyodik olarak tekrarlayan, şiddetli inflamasyon atakları görülür. Önerilen model ayrıca niçin proKaspaz 1 engelleyicilerinin FMF tedavisinde etkili olduğunu ancak CAPS'te olmadığını ve CAPS tedavisinde IL-1β'yı doğrudan engelleyen ilaçların kullanıldığını gösterir.

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ABBREVIATIONS

ASC	Apoptosis Associated Speck-like Domain Containing a CARD
APP	Acute Phase Protein
APR	Acute Phase Response
CAPS	Cryopyrin Associated Periodic Syndrome
CARD	Caspase-associated Recruitment Domain
CRP	C-reactive Protein
DAMP	Damage-associated Molecular Patterns
EP	Endogenous Pyrogen
ESR	Erythrocyte Sedimentation Rate
FCAS	Familial Cold Autoinflammatory Syndrome
IFN	Interferon
FMF	Familial Mediterranean Fever
IL	Interleukin
IL-1 RI	Interleukin-1 Receptor type I
IL-1 RII	Interleukin-1 Receptor type II
IL-1 Ra	Interleukin-1 Receptor Antagonist
IL-1 RAcP	Interleukin-1 Receptor Accessory Protein
LPS	Lipopolysaccharide
LRR	Leucine Rich Repeat
MEFV	Mediterranean Fever
MWS	Muckle-Wells Syndrome
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NLR	NOD-like Receptor
NLRP	NACHT, LRR and PYD domains-containing Protein
NLRP3	(Cryopyrin, CIAS1)
NOD	Nucleotide-Binding Oligomerization Domain (NACHT, PYRAF, NALP)
NOMID	Neonatal-onset Multisystem Inflammatory Disease (CINCA)
PAMP	Pathogen-associated Molecular Patterns
PGE	Prostaglandin E
PRR	Pattern Recognition Receptor
PYD	Pyrin Domain (DAPIN, PYRIN, PAAD)
SAA	Serum amyloid A
SIGIRR	Single immunoglobulin IL-1-related receptor
sIL-1 RI	Soluble Interleukin-1 Receptor type I
sIL-1 RII	Soluble Interleukin-1 Receptor type II
TLR	Toll-like Receptor
TNF	Tumor necrosis factor
TRAPS	Tumor Necrosis Factor Receptor Associated Periodic Syndrome
WT	Wild type

Chapter 1

INTRODUCTION

1.1 The Immune System

The immune system is the collection of various related cell types located in different parts of the organism. Immune cells have specialized immunity related functions, e.g., recognition of the non-self, signaling with other cells, learning and memory. These cells that form the immune system protect the body from bacterial, parasitic, viral and fungal infections (referred to as foreign antigens) and self antigens. Inflammation is the organisms' protective response to remove the insult and to initiate the healing. Inflammatory reaction triggered by the recognition of pathogen or danger associated molecular patterns usually results in the elimination of the stimuli and protection of the body integrity. However, when the insult level is higher than the immune system can handle or due to problems in the control mechanisms limiting the reaction against these harmful stimuli, immune disorders arise leading to some forms of discomfort, tissue or organ damage or even death, depending on the magnitude of the insult or the level of insufficiencies in the control mechanisms.

1.2 Autoinflammatory and Autoimmune Diseases

Autoinflammatory syndromes, also called periodic fever syndromes, are a class of disorders characterized by recurrent episodes of inflamed tissues such as joints, skin, gut and eyes, generally accompanied by fever, in the absence of any pathogen or auto-antibodies [6]. FMF, CAPS, Blau Syndrome and TRAPS are among the members of this disease class. A list of autoinflammatory syndromes, related genes, mode of inheritance, treatments, and other relevant information are available in [7].

Autoinflammatory syndromes are usually caused by the mutations within the cells regulating the innate immune system. Functions of innate immunity include:

- Physical barrier to infection in skin, lungs and mouth
- Recognition of the pathogens
- Inflammation through cytokines (small proteins that take part in cell signaling)
- Fever
- Activation of adaptive immunity

The innate immune system consists of neutrophils, macrophages, and natural killer cells, which are the first cells to respond to an infection. These cells can recognize common molecular patterns present in many types of pathogens. When activated, these cells normally secrete cytokines that trigger inflammation. With the initiation of the trigger signal, immune cells migrate to the infected area, attacking the invading pathogen. Mutations in innate immunity-related genes, cause unprovoked activation, i.e., increase in some specific proinflammatory cytokine (e.g. IL-1) concentration which triggers the immune system. The body reacts to the danger signals produced and acts as if there is an infectious agent.

Autoimmune diseases, on the other hand, are caused by dysregulations related with the adaptive immune system. The adaptive immune system, consisting of specialized B and T cells, is more sophisticated than the innate. In autoimmune diseases, the adaptive immune system produces antibodies to the antigens and then attacks the healthy tissue. A summarized comparison of autoinflammatory and autoimmune diseases are in Table 1.1.

	Autoinflammatory Diseases	Autoimmune Diseases
Immunological	Disruption of innate immune system	Disruption of adaptive immune system
Cells Involved	Neutrophils, macrophages	B and T cells
Clinical Recognition	Familial history, specific symptoms	MHC associations, autoantibodies
Therapy	Blocking cytokine cascades	Blocking T/B cells or their products

Table 1.1: Comparison of autoinflammatory and autoimmune diseases

Most of the work on the immune system have so far concentrated more on the second line defense, i.e., the adaptive immunity, since it is specific and considered as *more complicated*. However, understanding the behavior of the hereditary autoinflammatory syndromes, related with innate immunity, will not only provide improvements in the treatment of these diseases but also improve our understanding on the very basics of the immune system.

1.3 Mathematical Disease Modeling

The immune system is a complex system with a vast number of components. Quantitative approaches have recently been used in order to attain a better understanding of the immune system and immunity related diseases. Mathematicians have tried to understand how the immune cells interact with each other to constitute the total activitiy of the immune system. Mathematical models are of course not yet detailed enough to describe the quantitative behavior of all immune cells but the overall observed behaviors can be modeled using several well established tools of mathematics, biochemistry and more.

The results obtained from the theoretical immune system models may lead to identification of new tools for the treatment and prevention of immune system related diseases.

1.4 Thesis Outline

In Chapter 1, the immune system and two classes of immunity related diseases, namely autoimmune and autoinflammatory diseases, are introduced. Motivation for mathematical disease modeling is also given. In Chapter 2, a member of the autoinflammatory syndromes, Familial Mediterranean Fever, is examined. Known pathogenesis and pathophysiology and the key mediators are summarized and treatment options are presented. Chapter 3 concentrates on CAPS, which is usually a more severe case than FMF. Chapter 4 presents a unique mathematical model capturing key aspects of FMF and CAPS. Bifurcation analyses are performed on the model in addition to providing simulation results obtained with the model in Chapter 5. Chapter 6 concludes the thesis with a summary and possible future research directions.

Chapter 2

FAMILIAL MEDITERRANEAN FEVER (FMF)

2.1 Background Information

FMF is one of the autoinflammatory diseases associated with a mutation in the *MEFV* (MEditerranean FeVer) gene [8]. MEFV gene is located in the short arm of Chromosome 16 and encodes a 781-aminoacide protein called pyrin[1](marenostrin)[2]. FMF is inherited in an autosomal recessive fashion (although there are some reported exceptional heterozygotes manifesting FMF) and mostly affects people originating from the Mediterranean Sea (as the name suggests), e.g., mostly Armenians, Sephardic Jews, Cypriots, Turks and Arabs. The prevalence of FMF is 1/256 to 1/500 in non-Ashkenazi Jews and 1/1073 in the Turkish population; the carrier rate in Turkey is 1/5 [9].

The main symptoms are irregular attacks of fever (38-41°C), abdominal pain, chest pain and joint pain. The diagnosis criteria (Tel-Hashomer criteria) are summarized in Table 2.1. The duration of the flare (1-3 days) and remission (weeks or months) period varies considerably. In FMF patients, the acute phase response increases during the attack and decreases in the attack-free period. Although the acute phase response of FMF patients decreases in the attack free period, when compared with the healthy control, FMF patients' response is higher in general [4]. This serves as evidence to the persistent sub-clinical inflammation in the remission period where symptoms are not apparent. The acute phase response includes an increase in the white blood cell count, erythrocyte sedimentation rate, fibrinogen, C-reactive protein, serum amyloid A and phospholipase A_2 [10].

[1]Derived from *pyros*, meaning fever in Greek.

[2]*Mare nostrum* means Mediterranean Sea in Latin.

Diagnosis	Major Signs	Minor Signs
In addition to fever either one of the two:	Abdominal pain	Increased erythrocyte sedimentation rate
• $\geq$ 1 major signs and $\geq$ 1 minor signs	Chest pain	Leukocytosis
• $\geq$ 2 minor signs	Joint pain	Elevated serum concentration of fibrinogen
	Skin eruption	

Table 2.1: Simplified Diagnosis Criteria for FMF [1]

Although pyrin's structure and function have not been completely elucidated yet, it is clear that it has a role in the production of pro-inflammatory cytokines. Pyrin is expressed mainly in neutrophils, eosinophils, monocytes, dendritic cells and synovial and peritoneal fibroblasts (mostly in the innate immune system cells) [11] and regulates Caspase 1 activation. Caspase 1 is responsible for cleavage of proIL-1β and the subsequent release of bio-active IL-1β [12]. IL-1β is a very important pyrogenic cytokine in the inflammatory process. In FMF, over-stimulation of IL-1β production causes increased immune and febrile response. IL-1β's contribution in cell proliferation, differentiation and apoptosis is also in consideration. Excess IL-1β causes particular symptoms in particular organs.

2.2 Pathogenesis and Pathophysiology of FMF

Two proposed mechanisms in the action of pyrin on inflammation exist; namely, *Sequestration and Pyrin Inflammasome Hypotheses* [3]. *Sequestration Hypothesis* advocates that pyrin inhibits the action of Caspase 1 and IL-1β processing, portraying pyrin as an anti-inflammatory mediator.

In *Pyrin Inflammasome Hypothesis*, pyrin forms its own inflammasome resulting in more IL-1β activation, which makes pyrin a pro-inflammatory protein.

Following is an analysis of the pathogenesis of FMF.

2.2.1 Structure of Pyrin

Pyrin is composed of 5 domains [2]:

[3]Names of the hypotheses are taken from [13].

- PYRIN domain (PYD)
- bZIP transcription factor basic domain
- B-box zinc finger domain
- α-helical (coiled coil) domain
- B30.2 (PRYSPRY) domain

The structure is shown in Figure 2.1.

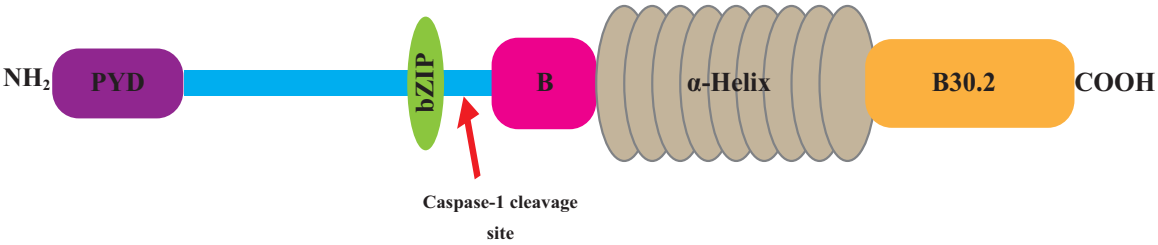


Figure 2.1: Pyrin Structure [2]

2.2.2 Cryopyrin Inflammasome in FMF

Pattern Recognition Receptors (PRR) are a class of innate immune response proteins that respond to Pathogen Associated Molecular Patterns (PAMP) or Damage Associated Molecular Patterns (DAMP). Localization and type of PRR vary considerably. Some subclasses of PRR are NOD Like Receptors (NLR) and Toll Like Receptors (TLR). PRR can identify either PAMP or DAMP and initiate the inflammatory response. DAMP are stress signals generated when an endogenous abnormality arises. Infectious dangers related with pathogens are signaled via PAMP provided that the cells are exposed to ATP.

PAMP include variety of non-self motifs associated with the pathogenic organisms. A typical example of PAMP is lipopolysaccharide (LPS), which is an endotoxin found on the outer membrane of Gram-negative bacteria. Some examples of other PAMP are nucleic acid variants such as double-stranded RNA (dsRNA), peptidoglycan and flagellin.

DAMP initiate immune response via non-infectious danger signals. Some examples of DAMP are heat shock proteins, tissue-injury related proteins, ATP, uric acid and host DNA.

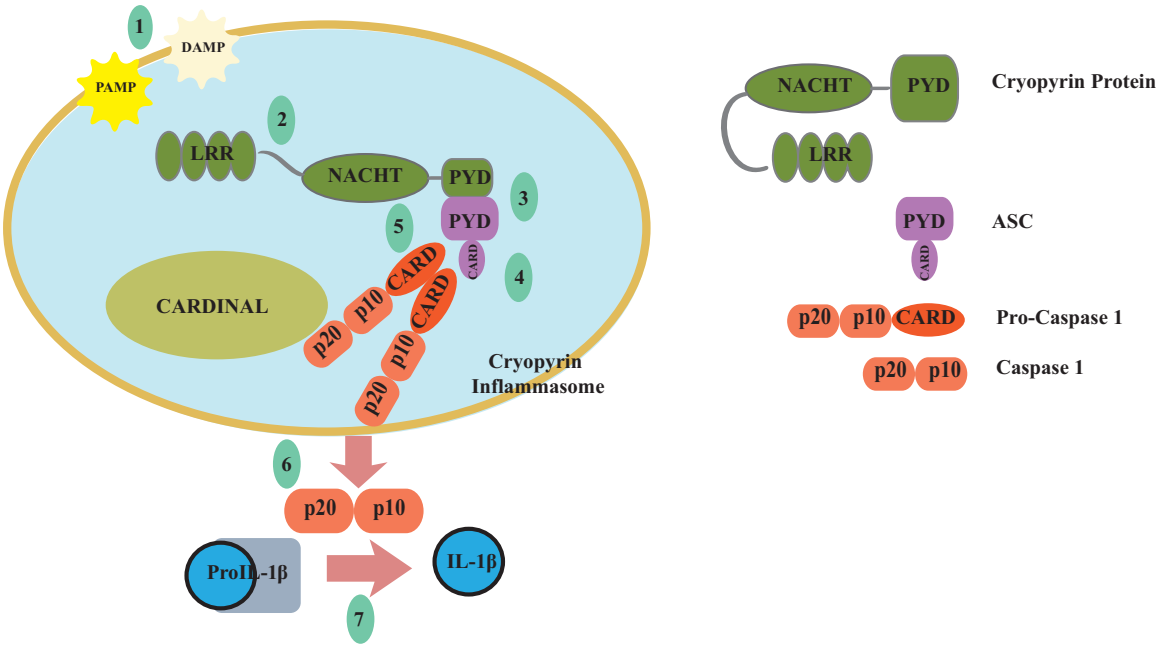
Inflammasome is a multiprotein oligomer composed of a sensor molecule (e.g. cryopyrin), an adaptor protein (ASC) and Caspase 1 which is responsible for activating the immune response. Inflammasome formation takes place, depending on the type of the inflammasome, in response to the specific PAMP or DAMP. NLR is a component of the inflammasome, having a crucial part in the immune response. In FMF, activated inflammasome yields Caspase 1 activation. Cryopyrin inflammasome, inflammasome of interest in FMF, is an inflammasome containing ASC, proCaspase 1, cryopyrin and cardinal proteins. This protein complex regulates and proteolytically activates important cytokines such as IL-1β through the activation of Caspase 1.

Cryopyrin protein is encoded by the NLRP3 gene and belong to NACHT-LRR (NLR) protein family. Like pyrin, cryopyrin protein also contains N terminal PYD that mediates its interaction with ASC. Cryopyrin can detect a variety of danger signals such as dsRNA, uric acid, bacterial ligands and imidazoquinolines. Although pyrin and cryopyrin share a similar N-terminal domain (PYD), the rest of the pyrin protein structure is different than cryopyrin.

We should emphasize here the differences between PYD, pyrin, cryopyrin (protein) and cryopyrin inflammasome. PYD is a common domain found in proteins such as pyrin, cryopyrin and other NLR proteins. This domain allows the interaction with other proteins containing PYD. For example, since ASC contains a PYD, pyrin and cryopyrin interact with ASC through these domains. Cryopyrin is an essential component of cryopyrin inflammasome.

Without any trigger (DAMP or PAMP), interaction between PYD of cryopyrin and ASC is not possible due to the structure of cryopyrin. With the presence of a stimulus, the interaction of the PYD domain of cryopyrin and ASC becomes possible. ASC interacts with the PYD of cryopyrin through its N terminal PYD. C terminal CARD domain of ASC is in interaction with the CARD domain of proCaspase 1. The cardinal brings another proCaspase 1 to the system. ProCaspase 1 molecules become closer to each other (induced-proximity-mediated-auto catalysis) which results in the proteolytic activation and release of p20 and p10 [2]. (Thus proCaspase 1 becomes activated and turns into Caspase 1.) ProIL-1β is a precursor which is proteolytically cleaved to a shorter, ac-

tive form, IL-1β, by Caspase 1. Figure 2.2 summarizes the inflammasome formation steps. Mature IL-1β molecules induce expression of other cytokines. Secondary cytokines such as IL-6 recruit immune cells to the site of infection. There is a balance between the levels of activation and inhibition of the inflammasome formation. Although IL-1β molecules help combat the infection, over-production of IL-1β causes adverse effects that are associated with immune diseases such as FMF and CAPS.



1: With DAMP or PAMP, inflammasome formation process is triggered.
2: A conformational change occurs in cryopyrin protein due to the trigger.
3: Interaction between PYDs of ASC and cryopyrin protein becomes possible after the conformational change.
4: CARD of ASC and proCaspase 1 interact.
5: Cardinal brings another proCaspase 1 to the system.
6: Induced proximity mediated auto catalysis results in the activation of Caspase 1.
7: Caspase 1 bio-activates IL-1β by cleavage.

Figure 2.2: Cryopyrin Inflammasome Formation

2.2.3 Caspase 1 Independent IL-1β Processing

Caspase 1 specifically cleaves 31-kd precursor proIL-1β to 17-kd biologically active IL-1β. Caspase 1 belongs to the caspase family of cysteine proteases. Some of the other members of this class, such as neutrophil serine proteases, proteinase 3, mast cell derived serine proteases [14], can also cleave proIL-1β. This suggests a redundancy in the mechanisms of IL-1β processing. According to [15], the role of other proteases is much more apparent in specific autoinflammatory syndromes such as rheumatoid arthiritis. Although in FMF and CAPS, over-production of IL-1β is mostly related to the high levels of Caspase 1 concentrations, we will also include the possible effect of other proteases in the model.

2.2.4 Maturation and Secretion of IL-1β

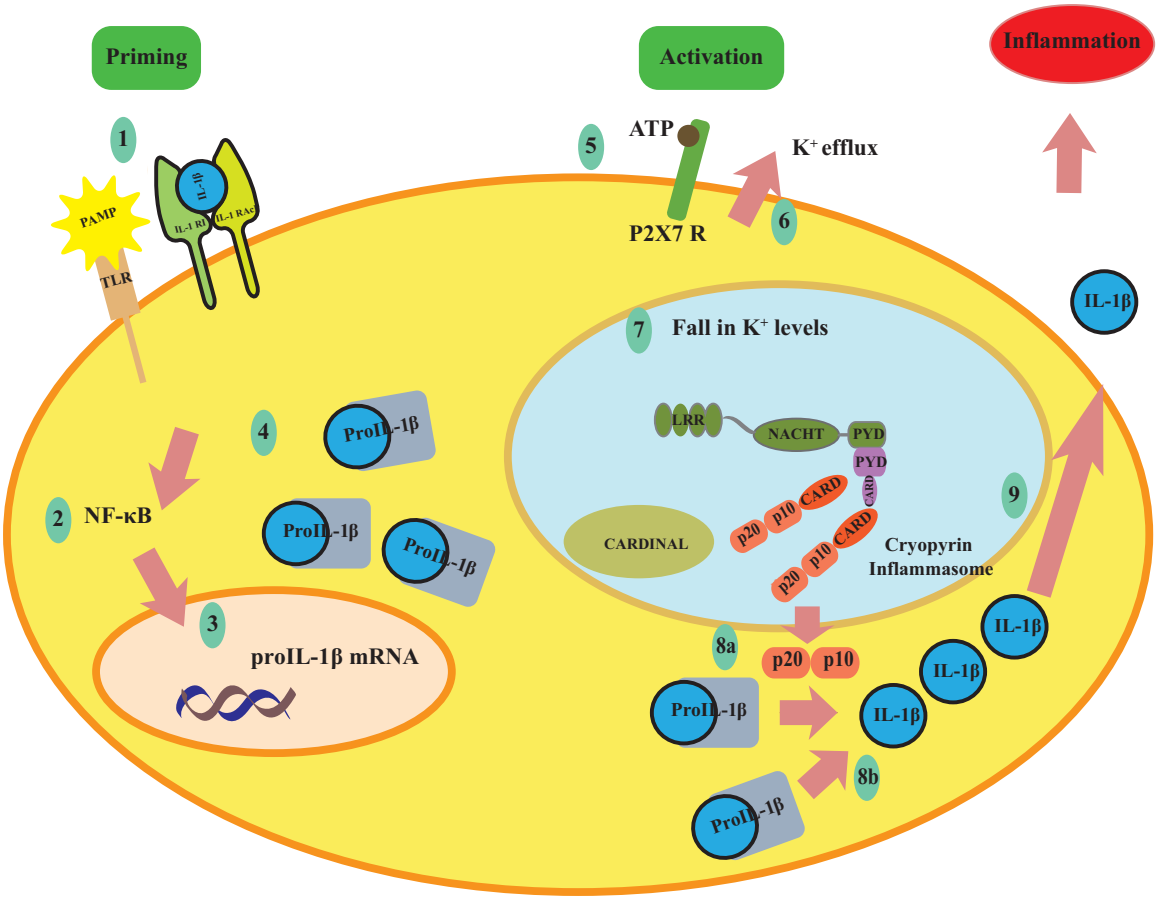
Maturation and secretion of IL-1β requires two distinct signals, priming and activation. With the priming signal, transcription of proIL-1β takes place. Activation signal results in the assembly of cryopyrin inflammasome, Caspase 1 activation and subsequently IL-1β release.

IL-1 RI is a member of TLR superfamily. Binding of mature IL-1β to IL-1 RI results in the downstream signaling of NF-κB pathway leading to the transcription of proIL-1β. TLR also recognize the microbial ligands and initiate proIL-1β transcription. Microbial ligands and endogenous cytokines are the priming signals. (Step 1 in Figure 2.3)

Activation signal (such as ion/membrane perturbations, ROS, pore-forming toxins, crystals and ATP) promotes the indirect activation of IL-1β secretion. In Figure 2.3, ATP activating cryopyrin inflammasome is shown as a representative example. Activation of P2X7 receptor by extracellular ATP results in the K^+ efflux. Fall in the intracellular K^+ levels triggers cryopyrin inflammasome formation. Inflammasome oligomerization leads to activation of Caspase 1, followed by the maturation and secretion of IL-1β. We should also note here the possible effect of other proteases (Please see Section 2.2.3).

In FMF patients, inflammasome activation is higher when compared to the healthy patients. Over-

activation of inflammasome causes over-production of IL-1β. This process is partially self-sustaining, i.e., IL-1β causes more IL-1β production (either via Caspase 1 or through Caspase 1 independent pathway) since IL-1β is one of the primary signals for IL-1β maturation and secretion.



Binding of ligand (PAMP or IL-1β) to TLR (**1**) activates NF-κB pathway (**2**). NF-κB signaling results in proIL-1β transcription and secretion (**3 & 4**). Binding of ATP to the P2X7 receptor is considered as the activation signal (**5**) which causes K^+ efflux (**6**) and subsequently a decrease in intracellular K^+ concentration (**7**). Together with PAMP or DAMP, fall in K^+ acts as the initiator of the inflammasome formation (**7**). ProIL-1β is cleaved by the product of the inflammasome, Caspase 1 (**8a**). However, Caspase 1 is not the only protease that can cleave proIL-1β(**8b**). Mature IL-1β is secreted and inflammation process is then started (**9**).

Figure 2.3: Maturation and Secretion of Il-1β Requires Two Signals

2.2.5 Sequestration Hypothesis

According to the Sequestration Hypothesis, pyrin inhibits Caspase 1 activation, therefore, it is an anti-inflammatory mediator. Mutations in this anti-inflammatory mediator cause problems in reg-

ulations of Caspase 1 and IL-1β. Some of the offered mechanisms on the contribution of pyrin in inhibiting Caspase 1 activation are listed here.

- ASC binds to pyrin through its PYD. Thus, ASC cannot participate in the formation of cryopyrin inflammasome. Since cryopyrin assembly does not take place, Caspase 1 is not activated, and hence IL-1β is not produced. (Step 1 in Figure 2.4)
- Due to the interactions between B30.2 domain of WT pyrin and the p20 and p10 domains of Caspase 1, pyrin can directly bind to both proCaspase 1 and Caspase 1 and hence indirectly prevent IL-1β activation. In FMF, the most common mutations (M608I, M694V, V726A) are generally in the C terminal B30.2 domain of pyrin. If there is a mutation in B30.2 domain of pyrin, the interaction between this domain and p20 and p10 subunits of Caspase 1 is diminished [16]. P20 and p10 form a heterodimer, i.e., Caspase 1 becomes activated. (Step 2 in Figure 2.4)
- Pyrin also interacts directly with proIL-1β which is an additional inhibition factor on IL-1β secretion.

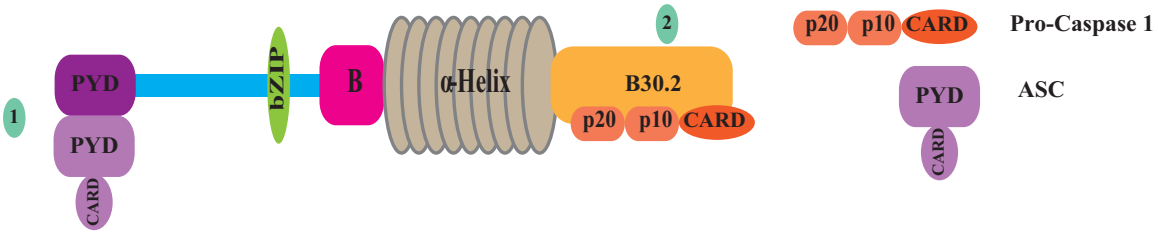


Figure 2.4: Anti-inflammatory Role of Pyrin

2.2.6 Pyrin Inflammasome Hypothesis

There is an ongoing debate on the anti- or pro-inflammatory role of pyrin. As opposed to the *Sequestration Model* in which it is proposed that pyrin inhibits Caspase 1 activation, there is an alternative model called the *Pyrin Inflammasome Model*.

The PYD of ASC interacts with the PYD of pyrin. With unidentified adapter protein, ASC, pyrin

and proCaspase 1 constitute an inflammasome, similar to cryopyrin inflammasome, resulting in the activation of Caspase 1 and IL-1β processing. The region where most of FMF mutations occur, B30.2 domain, is capable of sensing PAMP and DAMP [16]. Interaction of the danger signals with this domain results in binding of pyrin to ASC, i.e., pyrin inflammasome formation, thus IL-1β processing. This process is illustrated in Figure 2.5. In this model, FMF mutations are considered as *gain of function mutations* instead of *loss of function mutations*. With FMF related mutations, a broader range of PAMP can be sensed which becomes an evolutionary advantage. This view is fruitful in the explanation of the high allele frequencies for pyrin mutations in the population. In [17], it is suggested that C-terminal truncated version of pyrin results in increased Caspase 1 activation and IL-1β production. This result seems to contradict the *Pyrin Inflammasome Hypothesis*. However, it depends on whether C terminal truncated pyrin should be thought as active or inactive. Several reports indicate that deleting some domains may result in active molecules.

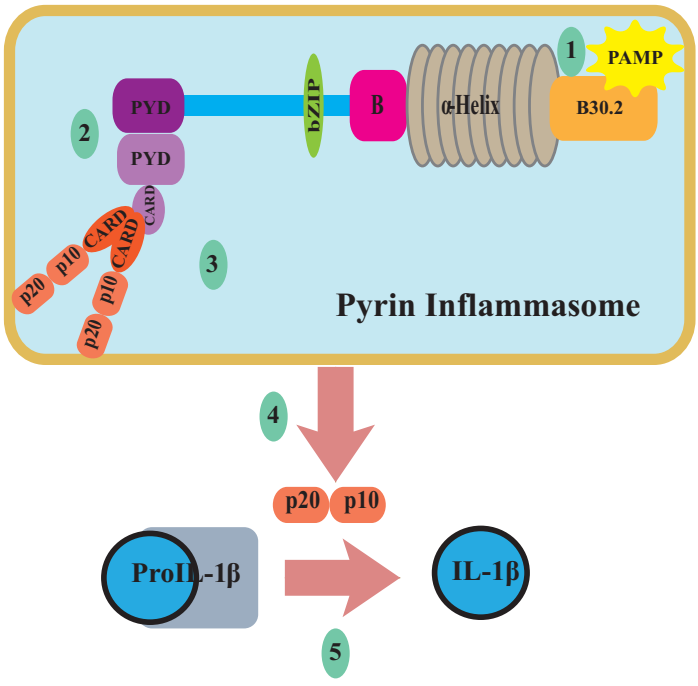


Figure 2.5: Inflammatory Role of Pyrin [3]

2.2.7 Evidence For Hypotheses

Pyrin Inflammasome Hypothesis

- When pyrin is transfected with ASC, Caspase 1 and IL-1β into 293T human embryonic kidney cells, pyrin activates Caspase 1 and IL-1β secretion [3].
- The interaction between Caspase 1 and WT and M694V mutated pyrin remains the same [18].
- Mutation in the B30.2 domain causes conformational changes in the oligomerization domain of the proteins, leading to more active inflammasomes. The relationship between mutation and inflammasome formation may be due to the decreased solubility properties of the mutant proteins. Reduction in solubility gives rise to more oligomer formation in mutants [3].

Sequestration Hypothesis

- Pyrin truncated mice demonstrate more Caspase 1 activation and IL-1β production in response to LPS when compared with WT pyrin [17].
- Ectopic silencing of pyrin in Th-1 human monocytes causes an increase in IL-1β secretion [2].
- Decreased interaction between mutated pyrin (M694V, M608I, V726A) and Caspase 1 results in more IL-1β secretion [16].

2.3 Cytokine Cascade in FMF

Cytokines are the main mediators of autoinflammatory diseases. The mechanism for the cytokine induction pathway is not fully clear. FMF occurs due to a mutation in pyrin, resulting in overproduction of IL-1β. However, not only IL-1β, but also IL-6 is also essential in attack and fever development in FMF. High levels of IL-1β cause an increase in IL-6. The increase in serum levels of IL-6 in the attack period is noteworthy and shown in Figure 2.6. IL-6 has both pro-inflammatory and anti-inflammatory roles. IL-6 changes the fever set point in the hypothalamus, responsible for the high fever in the attack period of FMF. Additionally, it mediates acute phase proteins. Levels of leukocyte, ESR, CRP, sIL-2 R, IL-6 and IL-10 increase considerably during an attack [19]. High levels of IL-6 may have suppressive effect in the production of other cytokines such as IL-1β and

TNF-α while activating the antagonist of IL-1β, IL-1 Ra [20].

Figure 2.6 shows the behavior of the levels of some classic markers of FMF (CRP, ESR, SAA,IL-6,TNF, IFN-γ). This chart does not represent the actual levels; instead the differences in the levels in remission and attacks and differences between healthy controls and FMF patients are emphasized. Although there is no attack in the remission period, the levels are higher in diseased patients than the healthy control. The step-wise increase in the levels suggests that even in attack-free state of FMF, subclinical inflammation persists. Non-classic markers (IL-8, IL-10, IL-12), on the other hand, do not show this behavior.

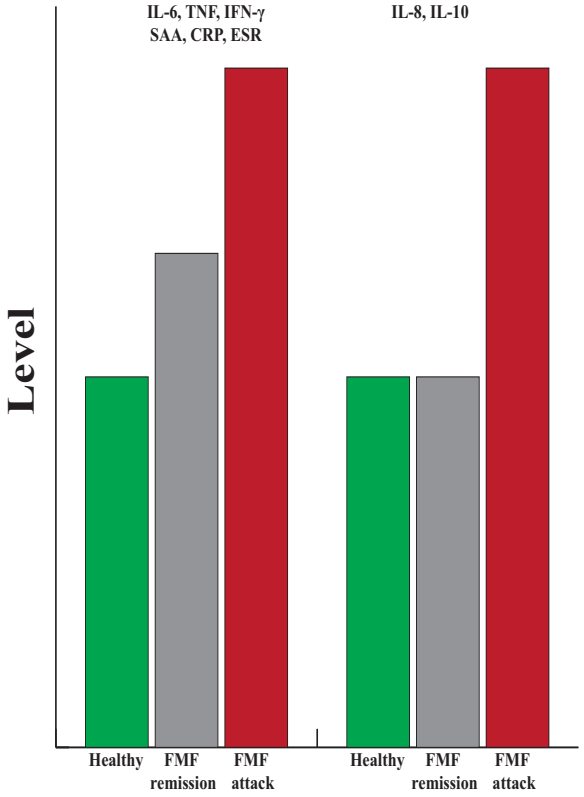


Figure 2.6: Classical and non-classical markers of FMF [4]

Although over-production of IL-1β is the main cause of FMF, surprisingly, a statistically significant increase in the IL-1β levels during the attack period cannot be detected in some of the measurements. Unchanged or even sometimes decreased levels of IL-1β may suggest new mechanisms for the disease or at least, different roles for IL-1β. The possibilities related to the function and detection

of IL-1β are listed here.

- Low plasma concentration(pg/ml) of IL-1β makes the detection difficult and error prone.
- Half life of IL-1β is short, therefore, detection may not be possible.
- IL-1β concentration increases before the acute phase and settles to the normal ranges when the patient enters the acute phase. Thus IL-1β acts as the initial trigger of inflammation [4].
- Both pre-cursor and active forms of IL-1β are measured with Elisa kits.
- Measurement of particular cytokine concentration depends on the levels of other cytokines in the medium. Dose dependence of IL-1β to another cytokine may lead to misinterpretations of cytokine production differences in control and diseased samples [21].
- Due to the oscillations in IL-1β levels in individuals with different patterns, statistically significant data cannot be obtained from the collection of data.

Despite the fact that some measurements do not support the increase in IL-1β levels during the attacks, over-production of IL-1β is considered as the most fundamental drawback of pyrin mutations, triggering the periodic bouts of fever and inflammation in FMF.

2.4 Complications and Treatment

The most severe complication of FMF is AA amyloidosis. In amyloidosis, normally soluble proteins become insoluble as a result of a change in the protein's secondary structure and proteins, which causes them to accumulate. Amyloidosis can cause severe problems, even renal failure. Disease onset is younger in patients with amyloidosis. Arthritis, erysipelas-like erythema manifestations are seen more frequently in patients with amyloidosis. FMF severity is related to the presence of amyloidosis [22]. AA amyloidosis develops in patients with specific MEFV gene haplotypes.

In the treatment of FMF, *colchicine* is a general standard which reduces the frequency of the attacks as well as delays amyloidosis development. The working mechanism of colchicine is not yet fully understood.

Secretion and over-production of IL-1β is the main problem in autoinflammatory syndromes. For this reason, mechanisms that inhibit IL-1β signaling are of interest. In FMF, one of the therapeutical implications is the use of IL-1 Ra, that will be described in Section 2.6. For patients who are colchicine intolerant or colchicine resistant, *Anakinra*, IL-1 Ra, is given. Efficacy of anakinra in FMF validates that IL-1β is the key cytokine in FMF pathogenesis. In other autoinflammatory diseases, various drugs inhibiting IL-1β action are given. Anti-IL-1β, IL-1 trap (rilonacept) and sIL-1 RI are some of the examples of IL-1β blocking strategies. Details of available IL-1β blockers will be described in Section 3.2.

2.5 Fever

Fever is the elevation of the body temperature above normal ranges (36.5 – 37.5°). During the attack period in FMF, high fever is one of the most disturbing symptoms felt by the patients. Endogenous and exogenous pyrogens account for the febrile response. LPS is an example of exogenous pyrogen whereas some of the cytokines are the endogenous pyrogens. In FMF, fever arises as an unprovoked episode, exogeneous pyrogens do not play a role, instead, cytokines are the key mediators. Cytokines (especially IL-1β) become activated by the immune cells and are secreted into circulation. Cytokines are activated as cascades and their effect on the progression of inflammation is redundant. Over-production of IL-1β triggers IL-6 production, which play a key role in FMF. Others are interleukin-8, TNF-β as well as IFN-α, IFN-β, and IFN-γ [23]. TNF-α also acts as a pyrogen, mediated by IL-1 release. These cytokines reach the brain and pass through the blood-brain barrier via active and passive transports. Cytokines bind to endothelial receptors and the arachidonic acid pathway is initiated. Subsequently, PGE_2, the ultimate mediator of the febrile response, is released. This causes an increase in the thermoregulatory set point in the hypothalamus. Although there are other mediators that affect the activation mechanism of the febrile response, this classical model captures the essence of the febrile response as summarized in Figure 2.7. Fever accompanies inflammatory response because it may help increase immune cell activity and stimulate some acute phase proteins. Therefore, fever is regarded as advantageous in fighting the infection and inflammation.

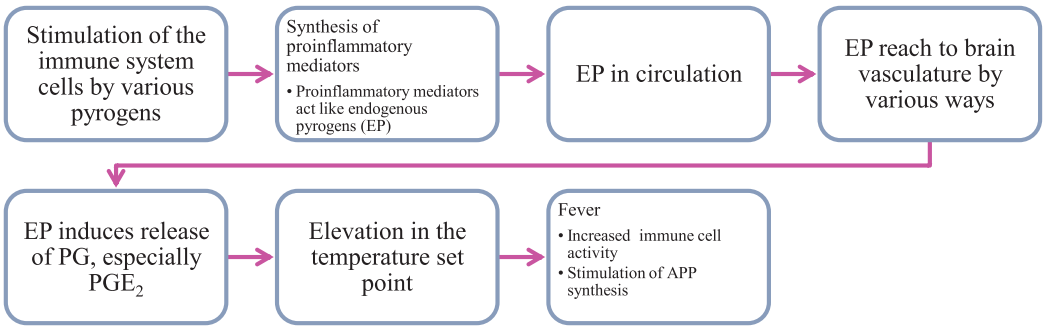


Figure 2.7: Cascade of Events Causing Fever [5]

2.6 Regulators of IL-1β Signaling

IL-1β is the most critical endogenous pyrogen in autoinflammatory diseases and its control is extremely important. Its receptor binding gives some clues regarding the possible mechanisms that may lead to end the active phase in FMF. There are natural inhibitors of IL-1 such as IL-1 Ra, IL-1 RII and other soluble receptors. In knock-out mice lacking IL-1 Ra, excessive inflammation has been observed. These mice develop spontaneous joint inflammation, vasculitis and skin inflammation [24]. The biological activities of IL-1 are initiated by binding of IL-1α and/or IL-1β to the same receptor, namely, IL-1 RI. IL-1 RI exists on the surface of a wide variety of cells. Binding of IL-1α and/or IL-1β causes a conformational change in IL-1 RI and recruits an accessory protein, IL-1 RAcP. Once IL-1/IL-1 RI/IL-1 RAcP complex is formed, signaling through other cascades such as NF-κB is activated. This is the only active form of this complex. Other scenarios fail to generate an active signal. Some of the cases that may cause no signal are listed here and shown in Figure 2.8.

- IL-1 Ra: Competes with IL-1β for binding to its receptor IL-1 RI and prevents binding of IL-1β and the subsequent downstream signaling.
- IL-1 RII: It is a decoy receptor similar to IL-1 RI. IL-1β binds to IL-1 RII instead of IL-1 RI. Cascades of other cytokines are not activated when IL-1β does not bind to IL-1 RI.
- SIGIRR: Prevents IL-1 RI/IL-1 RAcP heterodimerization.

- Soluble IL-1 RAcP: A soluble receptor that can bind to IL-1/IL-1 RI, but is incapable of propagating a signal. Enhances IL-1 binding to soluble IL-1 RII.
- Soluble IL-1 RI or RII: Soluble receptors that can bind to IL-1 and IL-1 RAcP, but are incapable of propagating a signal.

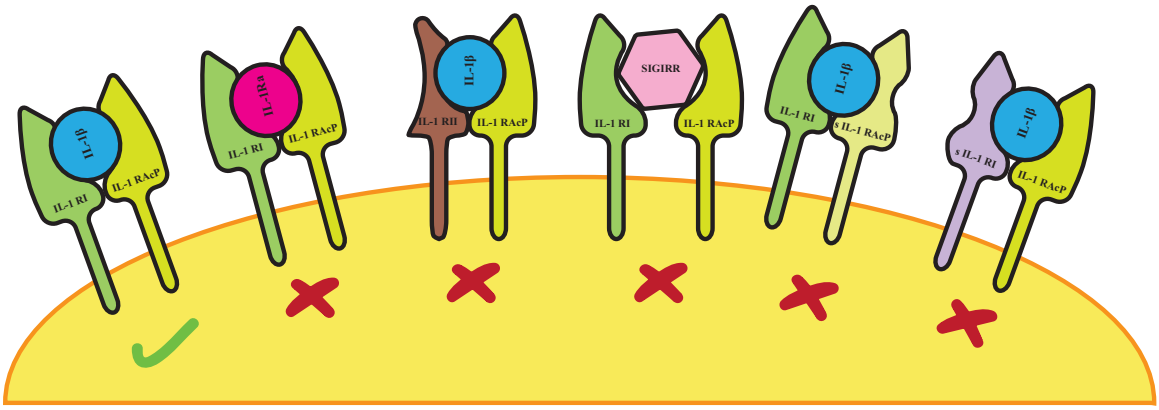


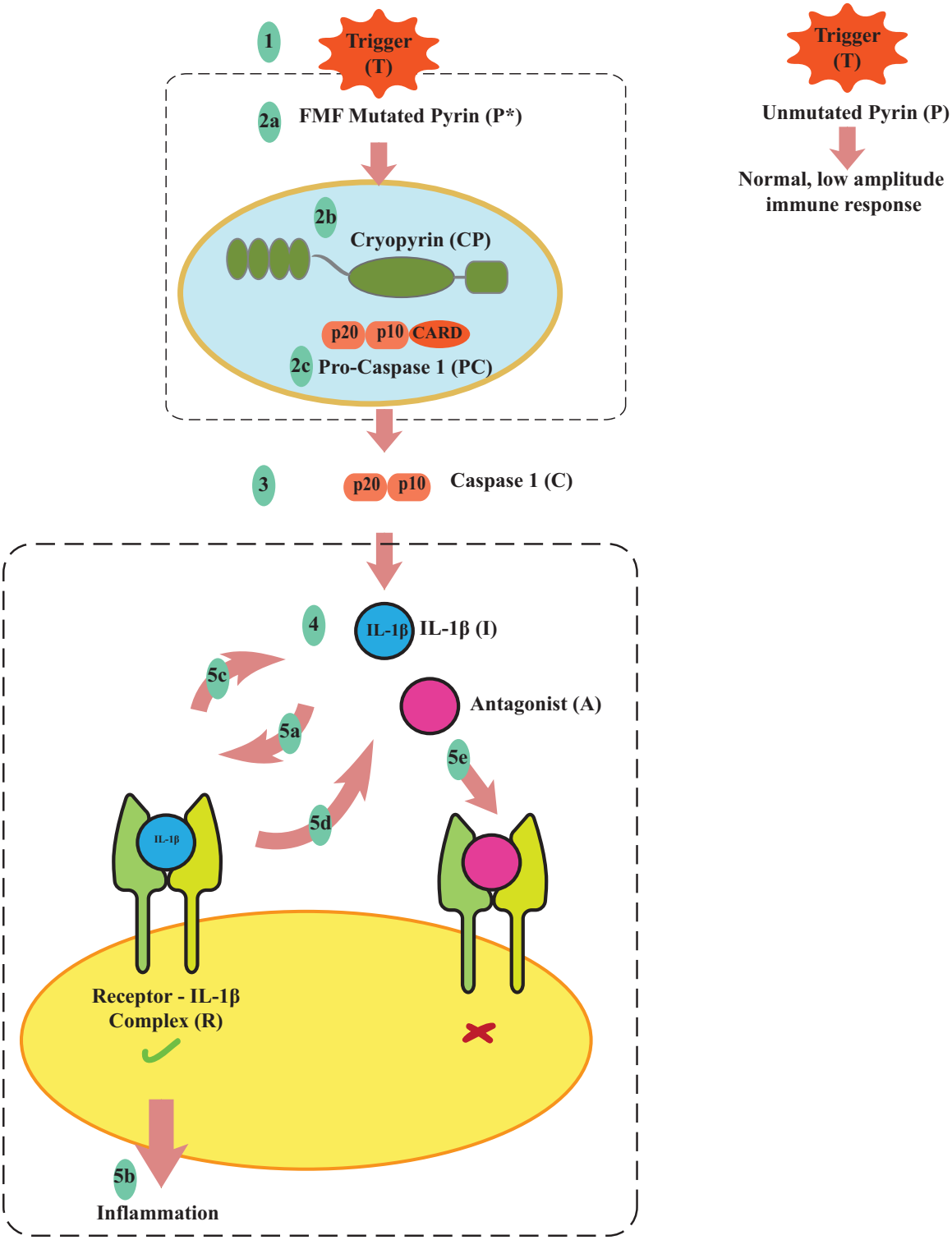
Figure 2.8: Mechanisms to Regulate IL-1β Signaling

The most remarkable aspect of autoinflammatory diseases is the periodic flare and remission cycles. These on/off durations and the severity of the disease is highly patient dependent. How this on/off switch operates (even what this switch is) is still not known. During the acute-attack period of the disease, IL-1 Ra levels [20] and sIL-1 RII levels [19] also increase compared to the levels before an attack. IL-1 Ra is induced by IL-1β-induced-NF-κB signaling which provides a negative feedback loop to control the inflammation [12]. These findings suggest that it is possible, even probable, that the increase in the natural inhibitors may neutralize the effect of IL-1β and this might be how the flare period ends.

2.7 FMF with a Modeling Perspective

From the two hypotheses presented on pyrin's role in inflammation in FMF in Section 2.2.5 and 2.2.6, there is more literature for the *Sequestration Hypothesis*. We will consider over-production of IL-1β as the driving cause of inflammation. PAMP and DAMP will be taken as triggers which activate inflammasome formation and the subsequent processes in pyrin mutants. When trigger is

not present, the disease is in its quiescent phase. The action of regulators of the IL-1β levels (IL-1 Ra, sIL-1 RII etc.) which were described in Section 2.6, will be unified and considered as one variable, the antagonist. Figure 2.9 illustrates the most crucial steps we have identified in FMF disease pathogenesis. Figure 2.9 and 3.2 form the foundation of the mathematical model that will be described in Chapter 4.



With Trigger, inflammasome formation process is triggered in pyrin mutants (**1** & **2a**). Cryopyrin protein and proCaspase 1 complex into an inflammasome due to nonfunctioning pyrin (**2b** & **2c**). ProCaspase 1 turns into Caspase 1 by the inflammasome action (**3**). IL-1β is maturated by Caspase 1 (**4**). IL-1β binds to the receptor (**5a**). IL-1β signaling cascade followed by inflammation is activated by the binding of IL-1β (**5b**). Signaling by the Receptor-IL-1β complex leads to further transcription of proIL-1β, resulting in an increase in IL-1β levels. Here, we had only considered the Caspase 1 independent processing of IL-1β (**5c**). Signaling by the Receptor-IL-1β complex also stimulates the antagonist production (**5d**). Binding of antagonist to the receptor does not result in active IL-1β signaling (**5e**).

Figure 2.9: Summary of the Pathogenesis of FMF

Chapter 3

CRYOPYRIN ASSOCIATED PERIODIC SYNDROMES (CAPS)

3.1 Background Information

CAPS comprises a spectrum of rare autoinflammatory syndromes; ranging from **FCAS** (Familial Cold Autoinflammatory Syndrome) and **MWS** (Muckle-Wells Syndrome) to **NOMID** (also called as CINCA) (Neonatal-Onset Multisystem Inflammatory Disease). These diseases are caused by autosomal dominant gain-of-function mutations in various domains of NLRP3 gene (also known as CIAS1 gene), located on chromosome 1 (1q44), which encodes a pyrin like protein, *cryopyrin, (NLRP3 protein)*. Approximately 100 different mutations (mostly missense mutations) in exon 3 of this gene, have been identified. Cryopyrin is expressed in monocytes, neutrophils and chondrocytes [25]. Cryopyrin is one of the NLR family proteins with a critical role in the regulation of the inflammatory response. Cryopyrinopathies lead to increased activity of a Caspase 1 activating inflammasome, i.e., cryopyrin inflammasome. The more inflammasome formation takes place , the more conversion of proIL-1β into IL-1β occurs. IL-1β not only activates fever pathway, but also causes pain sensitization, bone and cartilage destruction and activates acute phase response [26]. Although the initial steps of the pathogenesis of FMF and CAPS are different, they become quite similar in terms of the main cause and some of the design strategies in the treatment. IL-1 blocking therapies are applied to CAPS patients successfully. Similarities between FMF and CAPS will not be repeated in this chapter, only the differences will be indicated instead.

In Table 3.1, CAPS types and their symptoms are listed. The severity of the disease is also indicated.

Disease	Major Symptoms	Severity
FCAS	Cold-induced urticarial rash, and arthralgia Fever after exposure to cold	The mildest
MWS	Shared symptoms with FCAS and NOMID Inflammation is seen without provocation. Cold, stress and exercise also trigger the inflammation.	Intermediate
NOMID	Neonatal-onset high fever, persistent rash, aseptic meningitis, mental retardation, sensory deafness, papilledema, arthritis with bone over-growth, secondary amyloidosis	The most severe

Table 3.1: CAPS Types and Their Symptoms

3.2 Treatment Options

Many anti-inflammatories, anti-histamines and immunosuppressants have been tried for the treatment of CAPS. However, most of them do not control the disease or alleviate the clinical symptoms in the desired manner. This suggests that more targeted therapies are necessary. Since the main cause of CAPS is over-production of IL-1β, which should be targeted for effective treatment. IL-1 targeted treatment strategies for CAPS include:

- **Anakinra, IL-1β Receptor Antagonist:**
  Anakinra is a recombinant form of the natural IL-1 Ra and efficiently binds to IL-1 RI/IL-1 RAcP complex. The half-life is 4 to 6 h [27]. Several case reports demonstrated the efficacy of anakinra [28]. Although it is successful, daily injections are required due to the short half-life.

- **Rilonacept, Interleukin-1 Trap:**
  It is a dimeric fusion protein containing binding regions for IL-1 RI and IL-1 RAcP and fused to human IgG1. It was the first US-Food and Drug Administration-approved drug therapy for CAPS. The half-life is 8.6 days [29]. The cost, however, is higher when compared to anakinra.

- **Canakinumab (ACZ885, Novartis Pharma), IL-1β Monoclonal Antibody:**
  It is a human monoclonal antibody which is specific for only IL-1β. The half-life is 26 days and the optimized subcutaneous injection regime is every 8 weeks [30]. Canakinumab further relaxes the dosing schedule. Canakinumab has also FDA-approval for CAPS.

Figure 3.1 summarizes the treatment options.

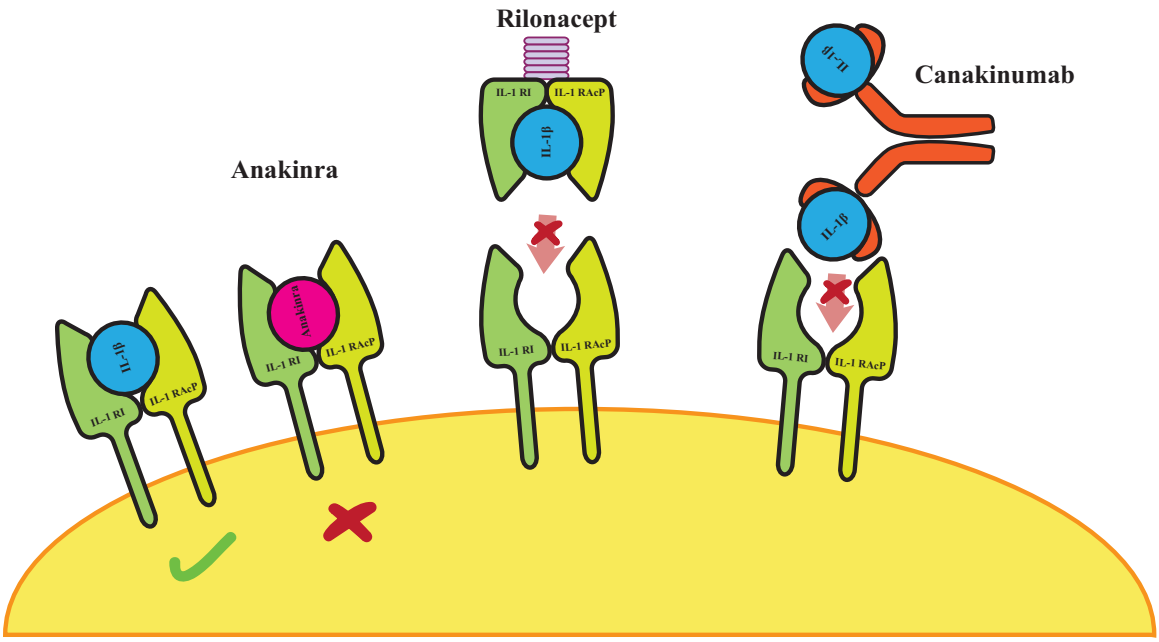


Figure 3.1: Treatment options for CAPS

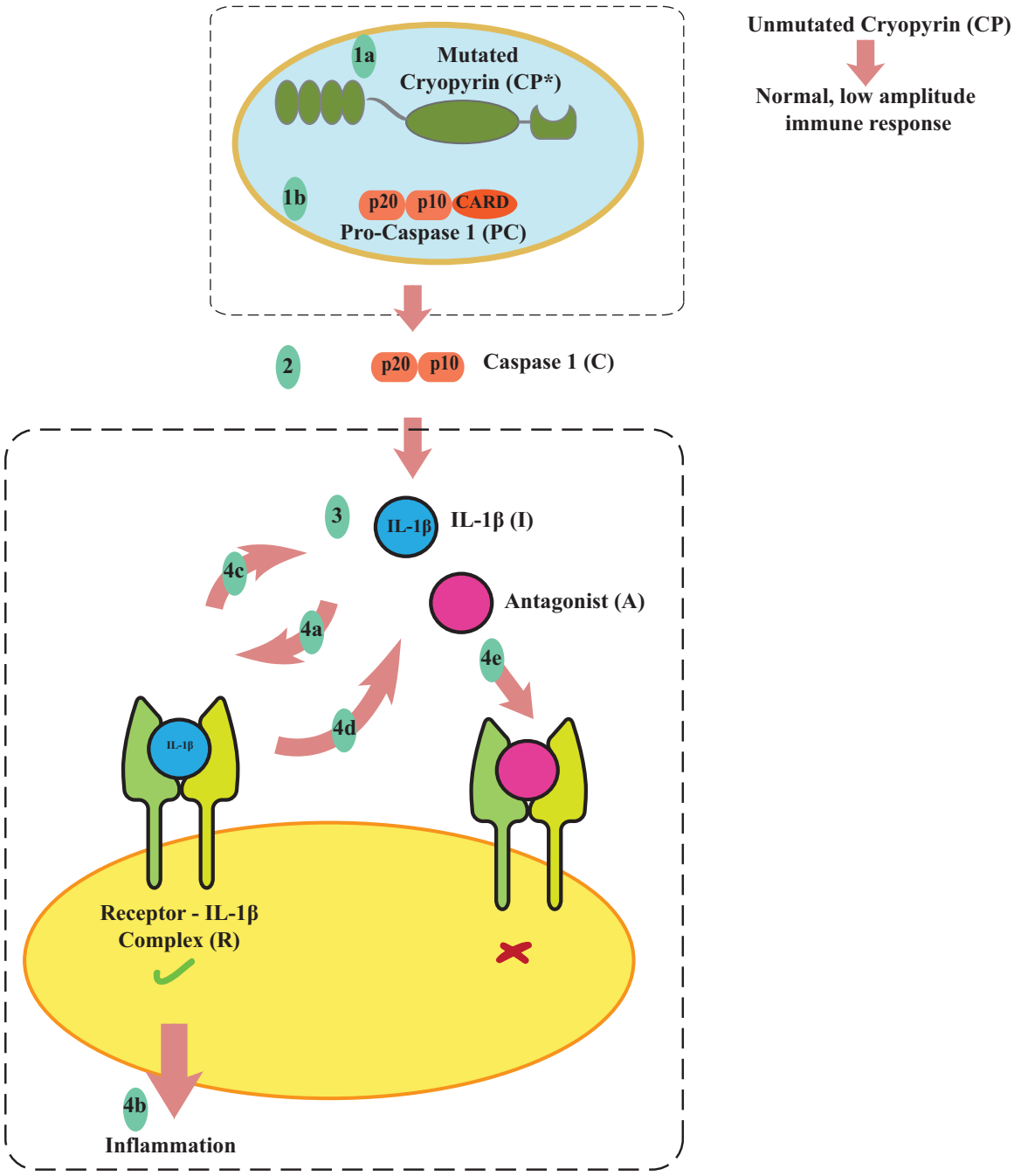
Colchicine, on the other hand, is not given to CAPS patients. Although the working mechanism is not understood, the efficacy of colchicine in FMF patients and almost no relieving action in CAPS patients suggest that colchicine has an inhibiting effect on proCaspase 1. Caspase 1 levels in CAPS patients are higher than the control and even FMF patients. Due to the mutation in cryopyrin, increased inflammasome activity results in a substantial decrease in proCaspase 1 levels (Please see Chapter 5). Since proCaspase 1 level is already low in CAPS patients, colchicine does not have a noticeable effect in treatment.

3.3 CAPS with a Modeling Perspective

Although CAPS attacks might occur following the initiation of a stimulus such as cold exposure in FCAS, no trigger is identified or associated in most disease attacks. This suggests that CAPS syndromes arise as recurrent episodes of fever in the absence of any insult.

Similar symptoms and characteristics between FCAS, MWS and NOMID suggest that it is possible to capture all of them in the same model.

Differences in the efficacy of colchicine in the treatment of FMF and CAPS patients suggest that Caspase 1 level is a critical parameter. If Caspase 1 level is high enough, even in the absence of any trigger, the flare and remission cycles are observed in CAPS patients.



Even without the trigger, inflammasome formation process takes place in cryopyrin mutants (**1a** & **1b**). ProCaspase 1 turns into Caspase 1 by the inflammasome action (**2**). IL-1β is maturated by Caspase 1 (**3**). IL-1β binds to the receptor (**4a**). IL-1β signaling cascade followed by inflammation is activated by the binding of IL-1β (**4b**). Signaling by the Receptor-IL-1β complex leads further transcription of proIL-1β and thus increase in Caspase 1 independent IL-1β levels (**4c**). Signaling by the Receptor-IL-1β complex also stimulates the antagonist production (**4d**). Binding of antagonist to the receptor does not result in active IL-1β signaling (**4e**).

Figure 3.2: Summary of the Pathogenesis of CAPS

Chapter 4

MODELING OF FMF AND CAPS

Modeling of infectious diseases has received considerable attention in the literature. Researchers from a wide range of fields such as immunology, epidemiology and physics to mathematics, have tried to understand the nature of the infectious diseases more systematically. Quantitative approaches to infection dynamics have been useful tool for developing better strategies for vaccines and other treatment options. The models for infectious diseases such as AIDS and HCV are well established [31].

Mathematical tools are used in various fields in biology. As more biologists become interested in system level studies, higher level models enable nonintuitive insights into biological studies.

The periodic nature of two autoinflammatory diseases, FMF and CAPS and the similarities in their pathogenesis as investigated in Chapter 2 and 3, are worth examining in more detail.

The external causes triggering FMF attacks are studied and tested throughly [32, 33]. CAPS flares, on the other hand, seems to be self sustaining, at least triggers for CAPS are not identified in the same extend as FMF. This suggests that FMF attacks occur as a result of an external trigger. Trigger (PAMP or DAMP) activates inflammasome formation and over-production of IL-1β due to malfunctioning of pyrin in FMF patients as described in Chapter 2. The immune response is then activated and the disease symptoms are observed in the attack period. When the trigger is deactivated by the immune action, disease enters a quiescent phase where most of the disease symptoms disappear. CAPS attacks, on the other hand, seem to be related to the dynamic properties of the key players' relative concentrations. Positive and negative feedback mechanims cannot keep these concentrations in normal ranges as a result of the mutation in cryopyrin, which causes periodic flare/remission cycles.

4.1 Positive and Negative Feedback Mechanisms

Positive and negative feedback loops are common regulatory motifs in biological signaling pathways. Genetic regulatory networks are one of the fields where mathematical modeling has been used successfully. Genetic regulatory networks include many cells that can interact in complex ways. Biological approaches alone are not adequate to resolve the dynamics. Mathematical models provide precise and explicit description of the interaction network and make it possible to predict the behavior of the system.

Homeostasis is an internal stable environment of organisms in which the variables are regulated and the internal conditions remain relatively constant. Normally, in response to a change in external conditions (e.g. change in temperature and acidity), the stability of the system is maintained. Negative and positive feedback mechanisms act like suppressors and amplifiers, maintaining homeostasis.

However when abnormalities are present in the organism (e.g. mutation in the negative or positive feedback loop elements), homeostasis cannot be sustained.

In FMF and CAPS, mutations in the regulator proteins (pyrin and cryopyrin) result in over-production of IL-1β, stimulating an inflammatory response. As pointed out in Sections 2.7 and 3.3, interactions between positive and negative feedback mechanims determine the overall behavior. Here, important positive feedback interactions that take role in both FMF and CAPS are listed.
Positive Feedback Mechanisms:

- Signaling by Receptor-IL-1β complex increases IL-1β levels, through further transcription of proIL-1β.
- Caspase 1 independent processing of IL-1β increases the active Receptor-IL-1β complex level.
- Receptor-IL-1β complex also increases the antagonist.

Negative Feedback Mechanism:

- Binding of the antagonist to the receptor does not result in IL-1β signaling, and hence de-

creases the active Receptor-IL-1β complex levels.

4.2 Enzyme Inhibition and Hill Kinetics

Enzymes' ability to convert can be affected by the substrate or other inhibitior molecules. Two kinds of enzymatic - competitive and noncompetitive inhibition reactions will be analyzed here. Enzymatic inhibitions result in alterations from the standard Michaelis-Menten form [34]. Each kind of inhibition results in different form of the rate equations. In each case, inhibition is assumed to be reversible and enzyme complexes are assumed to be in equilibrium. The reaction equations for competitive and noncompetitive inhibitions are derived as in [35].

In competitive inhibition, the inhibitor binds to the active site and prevents binding of the substrate. K_S and K_I are the dissociation constants. Competitive inhibition reaction equations are as follows, where E=enzyme, S= substrate, P=product, I=inhibitor:

$$\begin{aligned} &[\mathrm{E}] + [\mathrm{S}] \overset{K_S}{\rightleftharpoons} [\mathrm{E} \cdot S] \overset{k_1}{\longrightarrow} [\mathrm{E}] + [\mathrm{P}] \\ &[\mathrm{E}] + [\mathrm{I}] \overset{K_I}{\rightleftharpoons} [\mathrm{E} \cdot I] \end{aligned} \tag{4.1}$$

Competitive Inhibition Equations:

$$\begin{aligned} &K_S = \frac{[E][S]}{[E \cdot S]} \rightarrow [E \cdot S] = \frac{[E][S]}{K_S} \\ &K_I = \frac{[E][I]}{[E \cdot I]} \rightarrow [E \cdot I] = \frac{[E][I]}{K_I} \\ &[E_0] = [E] + [E \cdot S] + [E \cdot I] = [E]\left(1 + \frac{[S]}{K_S} + \frac{[I]}{K_I}\right) \\ &[E \cdot S] = \frac{[E_0]}{\left(1 + \frac{[S]}{K_S} + \frac{[I]}{K_I}\right)} \cdot \frac{[S]}{K_S} \\ &\frac{d[P]}{dt} = k_1[E \cdot S] \\ &\frac{d[P]}{dt} = \frac{V_{ci} \cdot \frac{[S]}{K_S}}{1 + \frac{[S]}{K_S} + \frac{[I]}{K_I}} \qquad (V_{ci} = k_1[E_0]) \end{aligned} \tag{4.2}$$

Binding of the inhibitior to the same site as the substrate results in a decrease in enzyme's affinity than that of the standard Michaelis-Menten. Since the substrate can outcompete for the binding sites, more substrate is required to achieve a higher reaction rate.

In noncompetitive inhibition, binding of the inhibitor reduces enzyme activity, but does not affect the binding of substrate. The inhibitor can bind to enzyme or enzyme-substrate complex. Also, the substrate can bind to the free enzyme and enzyme-inhibitor complex. Binding of one does not inhibit binding of the other. However, product cannot be formed if inhibitior is bound. The reaction equations are as follows:

$$\begin{aligned} &[\mathrm{E}] + [\mathrm{S}] \overset{K_S}{\rightleftharpoons} [\mathrm{E} \cdot S] \overset{k_2}{\rightarrow} [\mathrm{E}] + [\mathrm{P}] \\ &[\mathrm{E}] + [\mathrm{I}] \overset{K_I}{\rightleftharpoons} [\mathrm{E} \cdot I] + [\mathrm{S}] \overset{K_S}{\rightleftharpoons} [\mathrm{E} \cdot S \cdot I] \\ &[\mathrm{E} \cdot S] + [\mathrm{I}] \overset{K_I}{\rightleftharpoons} [\mathrm{E} \cdot S \cdot I] \end{aligned} \tag{4.3}$$

Non-competitive Inhibition Equations:

$$\begin{aligned} &K_S = \frac{[E][S]}{[E \cdot S]} = \frac{[E \cdot I][S]}{[E \cdot S \cdot I]} \rightarrow [E \cdot S] = \frac{[E][S]}{K_S}, [E \cdot S \cdot I] = \frac{[E \cdot I][S]}{K_S} \\ &K_I = \frac{[E][I]}{[E \cdot I]} = \frac{[E \cdot S][I]}{[E \cdot S \cdot I]} \rightarrow [E \cdot I] = \frac{[E][I]}{K_I}, [E \cdot S \cdot I] = \frac{[E \cdot S][I]}{K_I} \\ &[E \cdot S \cdot I] = \frac{[E] \cdot [I] \cdot [S]}{K_I K_S} \\ &[E_0] = [E] + [E \cdot S] + [E \cdot I] + [E \cdot S \cdot I] = [E]\left(1 + \frac{[S]}{K_S} + \frac{[I]}{K_I} + \frac{[S][I]}{K_S K_I}\right) \\ &\frac{d[P]}{dt} = k_2[E \cdot S] \\ &\frac{d[P]}{dt} = \frac{V_c \cdot \frac{[S]}{K_S}}{1 + \frac{[S]}{K_S} + \frac{[I]}{K_I} + \frac{[S][I]}{K_S K_I}} = \frac{V_{nci} \cdot \frac{[S]}{K_S}}{\left(1 + \frac{[S]}{K_S}\right)\left(1 + \frac{[I]}{K_I}\right)} \qquad (V_{nci} = k_2[E_0]) \end{aligned} \tag{4.4}$$

In large multimeric protein systems, cooperative binding is often seen. Especially, binding of inhibitor is generally cooperative. Cooperativity can be included in the enzymatic inhibition reactions as a Hill coefficient, n [36]. In positive (negative) cooperativity, binding of a ligand increases (decreases) the receptor's affinity and the likelihood of another ligand's binding also increases (de-

creases). Hill equation covers the cooperativity in ligand binding.

4.3 The Model

Motivated by the considerations above and in Sections 2.7 and 3.3, we construct a unifying disease model which captures both FMF and CAPS. The variables in the model are:

- Trigger (**T**): represents PAMP and DAMP which activates cryopyrin inflammasome formation.
- IL-1β (**I**): indicates the serum levels of IL-1β.
- Antagonist (**A**): represents the total activity that decreases the binding of IL-1β to its receptor. (e.g., Il-1 Ra, sIL-1 RII)
- Receptor (**R**): is used to indicate the amount of bound IL-1β, e.g., receptor-IL-1β complex.
- Caspase 1 (**C**): shows Caspase 1 levels.
- ProCaspase 1 (**PC**): demonstrates the free (not-pyrin bound) proCaspase 1 levels.
- Pyrin (**P**): represents the amount of pyrin. (Assumed to be constant.)

Explanation of the parameters used in the model are listed in Table 4.1.

The kinetic equations are given in (4.5) in the form of Ordinary Differential Equations (ODEs) that describe interactions of the key variables involved. The reaction system is composed as competitive and noncompetitive inhibition models used in biochemistry (Please see Section 4.2). We follow a similar approach in model development as in [37]. Here, to represent the interaction between **R**, **I** and **A**, we utilize competitive inhibition, due to the fact that the antagonist also binds to the same receptor and is structurally similar to the substrate, IL-1β. Inflammasome formation process is modeled based on noncompetitive inhibition reactions. ProCaspase 1 is considered as a substrate. Pyrin, on the other hand, inhibits the inflammasome formation by binding to **PC** (and **C**). The product of the process is **C**. Note that Hill effect is also taken into consideration in both inhibition reactions.

	Explanation
V_r	Maximum rate of **I** and **A** binding to the receptor
V_i	Strength of positive feedback
V_a	Strength of negative feedback
V_c	Maximum rate to form cryopyrin inflammasome
V_{pc}	Maximum rate of **PC** production
K_{ir}	Threshold for **I** to induce **R**
K_{ar}	Threshold for **A** to suppress **R**
K_{ri}	Threshold for **R** to induce **I**
K_{ra}	Threshold for **R** to induce **A**
K_{pcc}	Threshold for **PC** to induce **C**
K_{tc}	Threshold for **T** to induce **C**
K_{pc}	Threshold for **P** to suppress **C**
K_{ppc}	Threshold for **P** to suppress **PC**
k_{dr}	Degradation rate of **R**
k_{di}	Degradation rate of **I**
k_{da}	Degradation rate of **A**
k_{dc}	Degradation rate of **C**
k_{dpc}	Degradation rate of **PC**
k_{br}	Basal synthesis rate of **R**
k_{ba}	Basal synthesis rate of **A**
k_{bc}	Basal synthesis rate of **C**
k_{bpc}	Basal synthesis rate of **PC**
k_c	Conversion rate of **PC** into **C**
n	Hill function cooperativity exponent
α	Proportionality constant of **C** and **I**

Table 4.1: The parameters used in the model simulations

$$\frac{d[\mathbf{R}]}{dt} = V_r \cdot \frac{\left(\frac{[\mathbf{I}]}{K_{ir}}\right)^n}{1 + \left(\frac{[\mathbf{I}]}{K_{ir}}\right)^n + \left(\frac{[\mathbf{A}]}{K_{ar}}\right)^n} - k_{dr} \cdot [\mathbf{R}] + k_{br} \tag{4.5a}$$

$$\frac{d[\mathbf{I}]}{dt} = V_i \cdot \frac{\left(\frac{[\mathbf{R}]}{K_{ri}}\right)^n}{1 + \left(\frac{[\mathbf{R}]}{K_{ri}}\right)^n} - k_{di} \cdot [\mathbf{I}] + \alpha \cdot [\mathbf{C}] \tag{4.5b}$$

$$\frac{d[\mathbf{A}]}{dt} = V_a \cdot \frac{\left(\frac{[\mathbf{R}]}{K_{ra}}\right)^n}{1 + \left(\frac{[\mathbf{R}]}{K_{ra}}\right)^n} - k_{da} \cdot [\mathbf{A}] + k_{ba} \tag{4.5c}$$

$$\frac{d[\mathbf{C}]}{dt} = V_c \cdot \frac{\left(\frac{[\mathbf{PC}]}{K_{pcc}}\right)^n}{1 + \left(\frac{[\mathbf{PC}]}{K_{pcc}}\right)^n} \cdot \frac{\left(\frac{[\mathbf{T}]}{K_{tc}}\right)^n}{1 + \left(\frac{[\mathbf{T}]}{K_{tc}}\right)^n} \cdot \frac{1}{1 + \left(\frac{[\mathbf{P}]}{K_{pc}}\right)^n} - k_{dc} \cdot [\mathbf{C}] + k_{bc} \tag{4.5d}$$

$$\frac{d[\mathbf{PC}]}{dt} = V_{pc} \cdot \frac{1}{1 + \left(\frac{[\mathbf{P}]}{K_{ppc}}\right)^n} - k_c \cdot [\mathbf{C}] - k_{dpc} \cdot [\mathbf{PC}] + k_{bpc} \tag{4.5e}$$

For all the variables (**R**, **I**, **A**, **C** and **PC**) we have assumed a basal synthesis rate. Since the production rate of **I** is directly related to the **C** concentration according to our model, basal synthesis rate of **I** is not written explicitly, i.e., k_{bi} does not appear in Equation 4.5g. The rate of degradation is assumed to be proportional to the concentration of that variable.

I being the substrate and **A** being the inhibitior, Equation 4.5f captures competitive inhibition reactions. Equation 4.5g represents the effect of Caspase 1 independent positive feedback mechanism between **R** and **I**. Activation of **A** by **R** is captured in Equation 4.5h.

C, on the other hand, is generated as a result of a noncompetitive binding reaction as represented by Equation 4.5a. Here, binding is not used in a strict sense, instead it captures the possible interactions. With the initiation of **T**, **PC** forms the inflammasome at a maximal rate of V_c. **P** decreases inflammasome formation since it binds to **PC** and **C**. In inflammasome formation, other proteins such as cryopyrin, ASC and cardinal concentrations are taken as constant and not represented explicitly. Conversion of **PC** into **C** indicates the formation of inflammasome. Decrease in **PC** levels due to conversion to **C**, is captured in Equation 4.5b with a rate constant k_c. **C** affects the **I** concentration, proportional to an abstract constant α.

This model captures *Healthy*, *FMF* and *CAPS* in a unified manner. In *Healthy* mode, (arbitrarily chosen) normal values of the parameters that will be listed in Chapter 5 in Table 5.1 are used. *FMF* is a result of a mutation in pyrin protein. This mutation is attributed to an increase in the threshold for **P** to suppress **C** and **PC**, i.e., K_{pc} and K_{ppc} is increased to 10 from 1. *CAPS* is associated with a mutation in cryopyrin and subsequently more inflammasome formation. This mutation is captured by the V_c term. The strength of the inflammasome formation is increased to 450 from 1.15. Since we only try to observe the effect of the mutations, other parameters are fixed. Although the parameters used in the model do not have a direct physical equivalence, they become pivotal to reflect the observed behavior as will be seen in Chapter 5.

4.4 The Model as a Collection of Two Separable Subsystems

The model can be separated into two distinct subsystems as shown in Figure 4.1. Subsystem 1 is composed of noncompetitive inhibition reactions between **PC**, **T**, **P** and **C** and determines the amount of Caspase 1. These interactions are captured by Equations 4.5a and 4.5b. Proportional to constant α, **C** concentration contributes to production of **I**. Competitive binding reactions between **R**, **I** and **A** (subsystem 2) is independent of the subsystem 1 except for the **C** effect in **I**. These interactions are represented by Equations 4.5f, 4.5g and 4.5h. Given as an input to subsystem 2, two-subsystem model suggests that Caspase 1 level is the most critical parameter of the total system.

Depending on the amount of Caspase 1 level, subsystem 2 exhibits varying characteristics, i.e., monostability, excitability and oscillatory behavior. These behaviors correspond to three distinct modes of the model which are *Healthy*, *FMF* and *CAPS*, respectively.

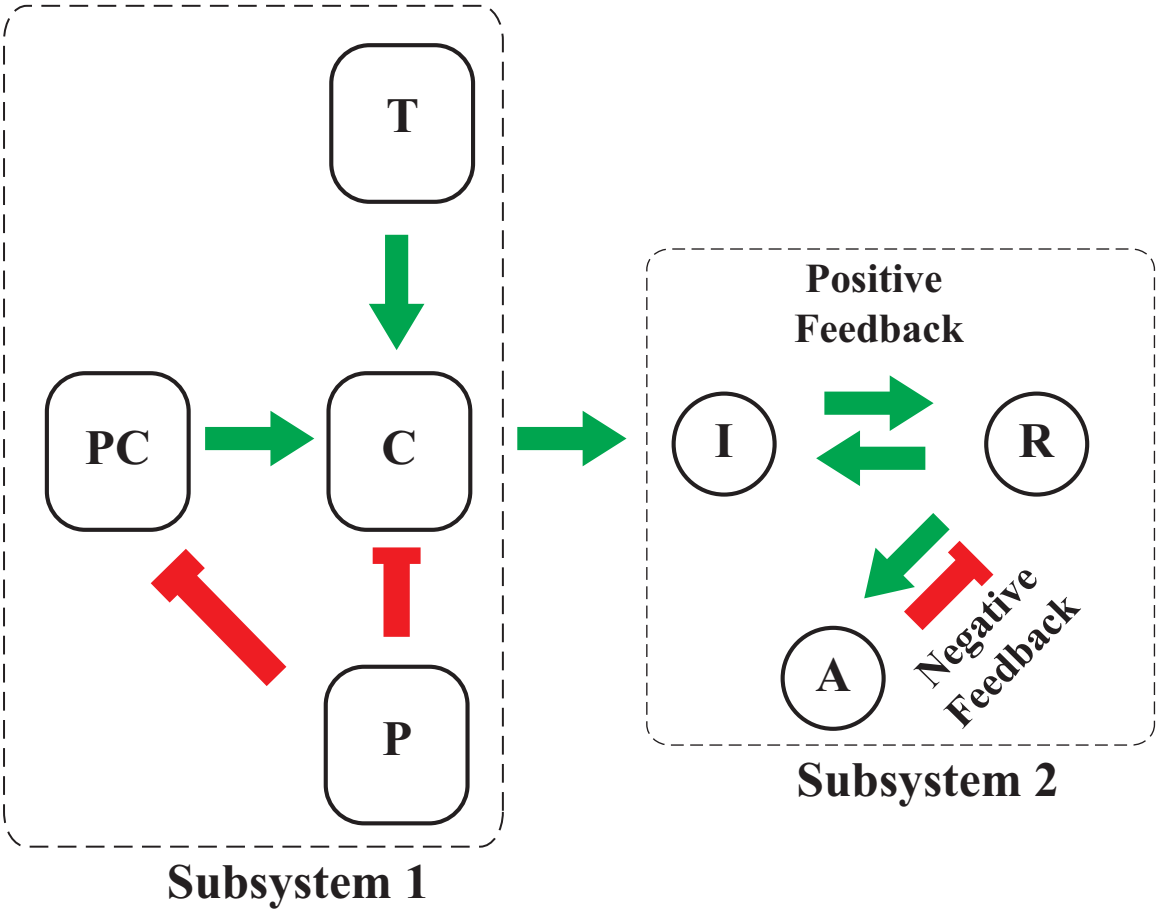


Figure 4.1: The Model as a Collection of Two Separable Subsystems

The direction of each green(red) arrow represents increasing(decreasing) effect.

4.5 Bifurcation Analyses

Three different behaviors, *Healthy*, *FMF* and *CAPS*, are revealed here with bifurcation analyses. Since Caspase 1 level is considered as the most critical component of the total system (see Section 4.4), it is selected as the bifurcation parameter. Caspase 1, being the output of subsystem 1, has constant fixed value, i.e., subsystem 1 has one steady state solution. This enables uncoupling of subsytem 1 and subsystem 2. We have performed bifurcation analyses on subsystem 2 by considering **C** as a parameter. Each **C** value corresponds to a different parameter regime of subsytem 1.

In Figure 4.2, stable steady state solutions are shown as a solid line and the unstable ones are represented by red dashed lines. Periodic stable (unstable) solutions are shown by green solid (blue dashed) lines. Note that bifurcation diagrams of **R**, **I** and **A** follow the same stability pattern. In low value (**C** = 1 to **C** = 1.44) region of **C**, there is one stable steady state solution. Increase in low value **C** region, does not cause drastic changes in **R**, **I** and **A**. In monostability region, **C** values always stay in this region, independent of the duration and magnitude of **T**. In excitable mode, however, when **T** is high, **C** jumps into the region (**C** = 1.44 to **C** = 1.48) where there is one stable and two unstable solutions. **R**, **I** and **A** values increase considerably. If **T** turns out to low values, **C** moves back to the low value region. When **C** is increased further (**C** = 1.48 to **C** = 1.9), the complex conjugate eigenvalue pair moves away from the imaginary axis. Oscillatory mode corresponds to this region where there is one stable periodic solution and an unstable solution. A pair of complex conjugate eigenvalues cross the imaginary axis, corresponding to Hopf bifurcation. In this mode, oscillations (limit cycle) in **R**, **I** and **A** become apparent. The maximum levels of **R**, **I** and **A** are the highest amongst other modes. Going beyond further than **C** = 1.9 is physiologically unlikely.

Numerical bifurcation analyses in Figure 4.2 are performed by using XPPAUT software package [38]. Stability of the steady state solutions are tested using MATLAB [39].

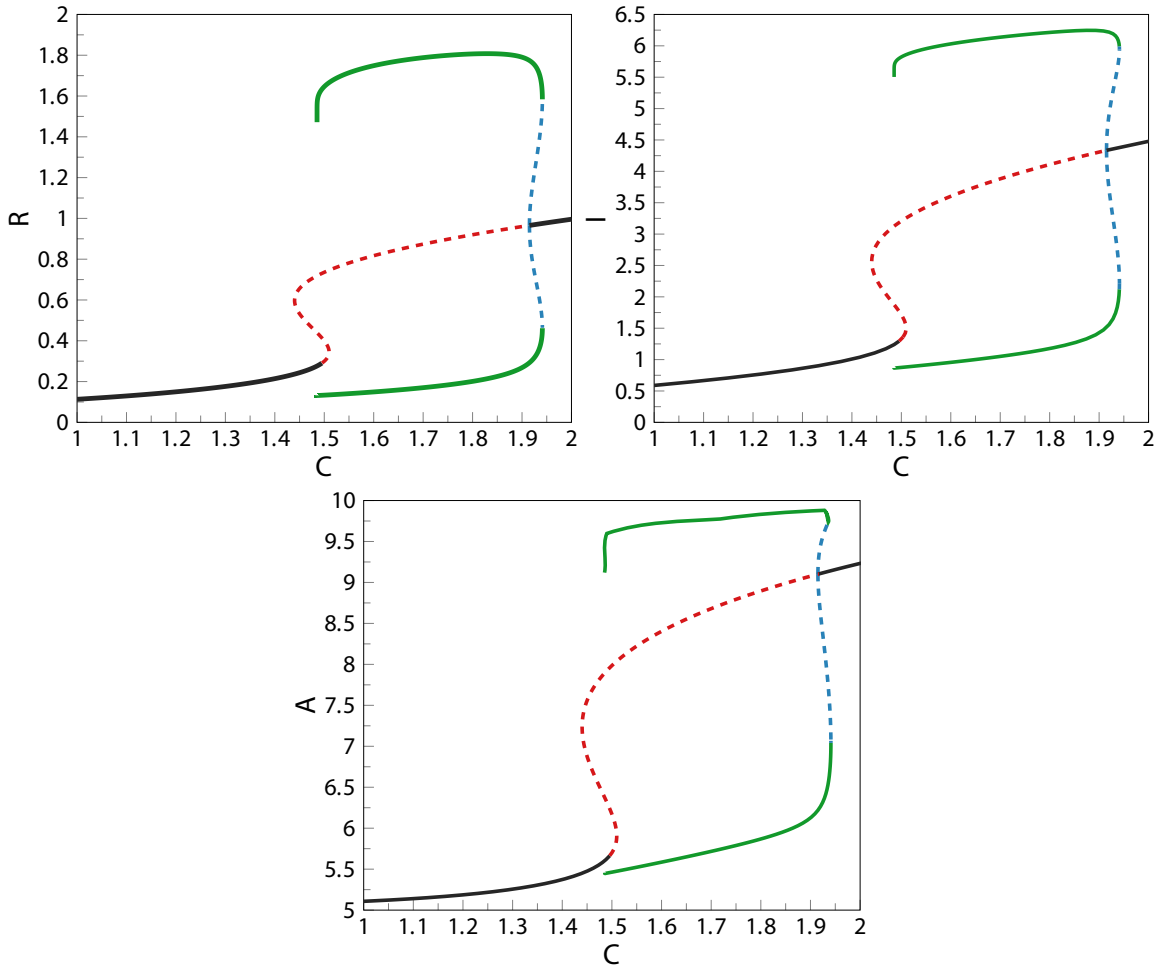


Figure 4.2: Bifurcation Analyses

Stable (unstable) steady state solutions are shown as a solid black (red dashed) line. Periodic stable (unstable) solutions are represented by green solid (blue dashed) lines.

Chapter 5

RESULTS

The parameter values used in time simulations in each mode are listed in Table 5.1. Only the changed parameters are noted for *FMF* and *CAPS*.

Parameter	*Healthy*	*FMF*	*CAPS*
V_r	1		
V_i	1.4		
V_a	0.17		
V_c	1.15		450
V_{pc}	1		
K_{ir}	1		
K_{ar}	1		
K_{ri}	1		
K_{ra}	1		
K_{pcc}	1		
K_{tc}	1		
K_{pc}	1	10	
K_{ppc}	1	10	
k_{dr}	0.2		
k_{di}	0.2		
k_{da}	0.02		
k_{dc}	10		
k_{dpc}	0.5		
k_{br}	0.01		
k_{ba}	0.1		
k_{bc}	1		
k_{bpc}	1		
k_c	0.3		
n	2		
α	0.1		
P	2		

Table 5.1: The parameter values used in the model simulations

In this chapter, time courses of **R**, **I**, **A**, **PC** and **C** in response to a pulse input in *Healthy* and *FMF* modes and a constant trigger in *CAPS* are shown. In *Healthy* and *FMF* modes, magnitude of **T** is stepped from 0.1 to 0.94 (and back to 0.1). The trigger is applied at time=100 and duration of **T** is set to 150. In *CAPS*, a constant trigger with **T** = 0.1 is applied, with the dashed line representing **T**. With the presence of a pulse trigger, there is a slight increase in **R**, in *Healthy* mode. The strength of

R is not enough to activate the cytokine cascade for inflammation. In *FMF*, **R** values over-exceeds *Healthy*, initiating the inflammation. When trigger disappears, **R** values turn into normal ranges in a while. High **R** corresponds to the flare (attack) period in FMF. As long as the trigger is not present, remission period continues. Depending on the magnitude and duration of trigger, attacks occur or do not occur. In *CAPS*, even though **T** is not present (**T** = 0.1), **R** levels oscillate due to the dynamics of the interaction between **R**, **I** and **A**. The maximum level is higher than that of FMF. As proposed by our model, CAPS patients follow this oscillatory flare/remission pattern. **I** and **A** follow the same behavior with **R**.

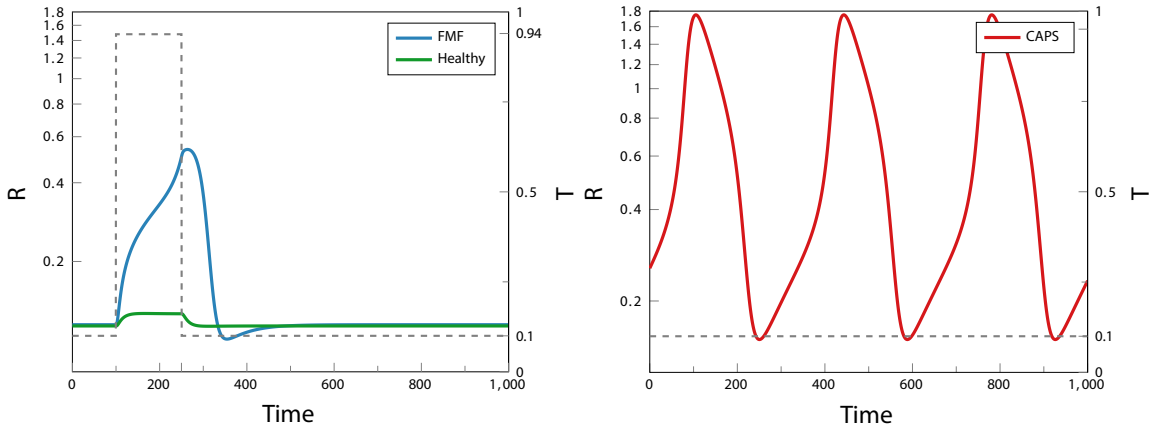


Figure 5.1: **R** Levels in *Healthy*, *FMF* and *CAPS*

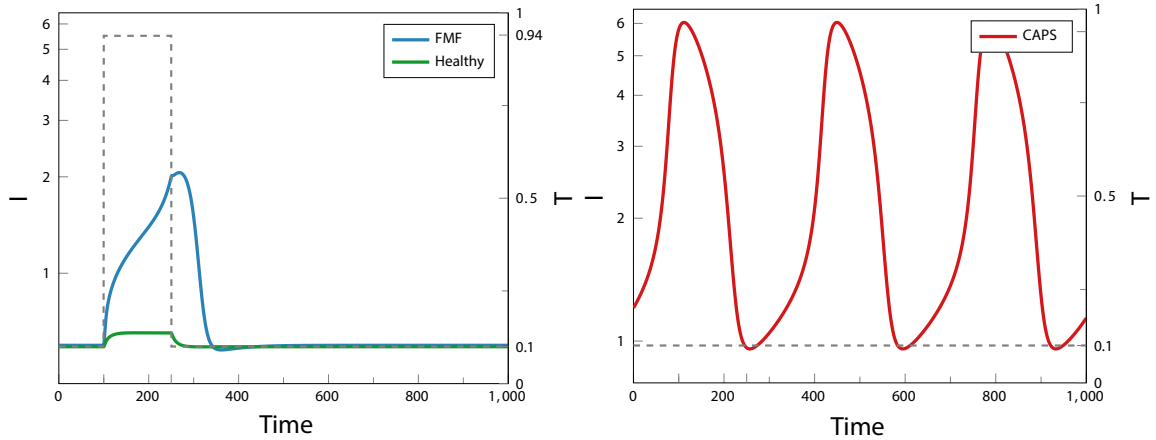


Figure 5.2: **I** Levels in *Healthy*, *FMF* and *CAPS*

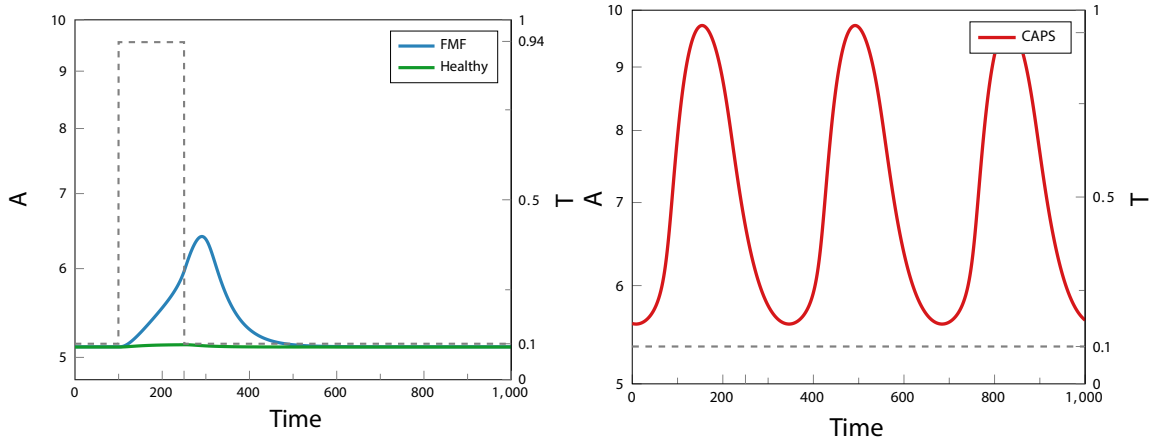


Figure 5.3: **A** Levels in *Healthy*, *FMF* and *CAPS*

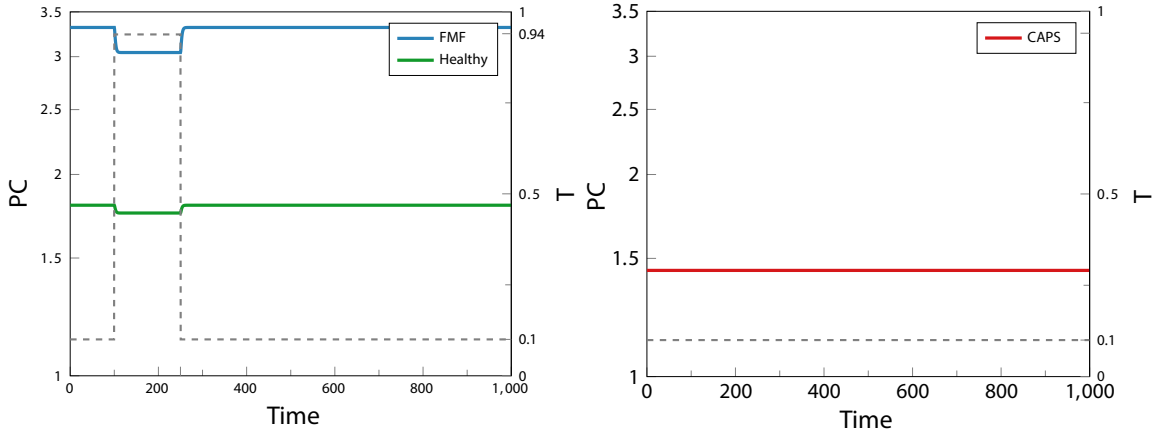


Figure 5.4: **PC** Levels in *Healthy*, *FMF* and *CAPS*

With the presence of **T**, proCaspase 1 is converted to Caspase 1 through cryopyrin inflammasome formation. Due to the mutation in pyrin in *FMF*, pyrin cannot suppress the inflammasome formation by binding to the **PC**. Figure 5.4 shows the free (not pyrin-bound) **PC** levels in *Healthy*, *FMF* and *CAPS*. In *FMF*, **PC** level is the highest due to the mutation in pyrin. In *CAPS*, as a result of a cryopyrin mutation, conversion of **PC** to **C** is more favored. Therefore, **PC** is the lowest in *CAPS*. Although FMF and CAPS are similar diseases in terms of over-production of IL-1β, target of drugs used in the treatment is different. IL-1β blocking strategies are the general approach in CAPS, while *colchicine* is the gold standard to reduce FMF flares. Ineffectiveness of colchicine in CAPS suggests

that colchicine may have an effect only in a specific **PC** range. Low levels of **PC** may correspond to the ineffective range of colchicine. Although the mechanism of colchicine is not known yet, colchicine suppresses cryopyrin inflammasome activation [40].

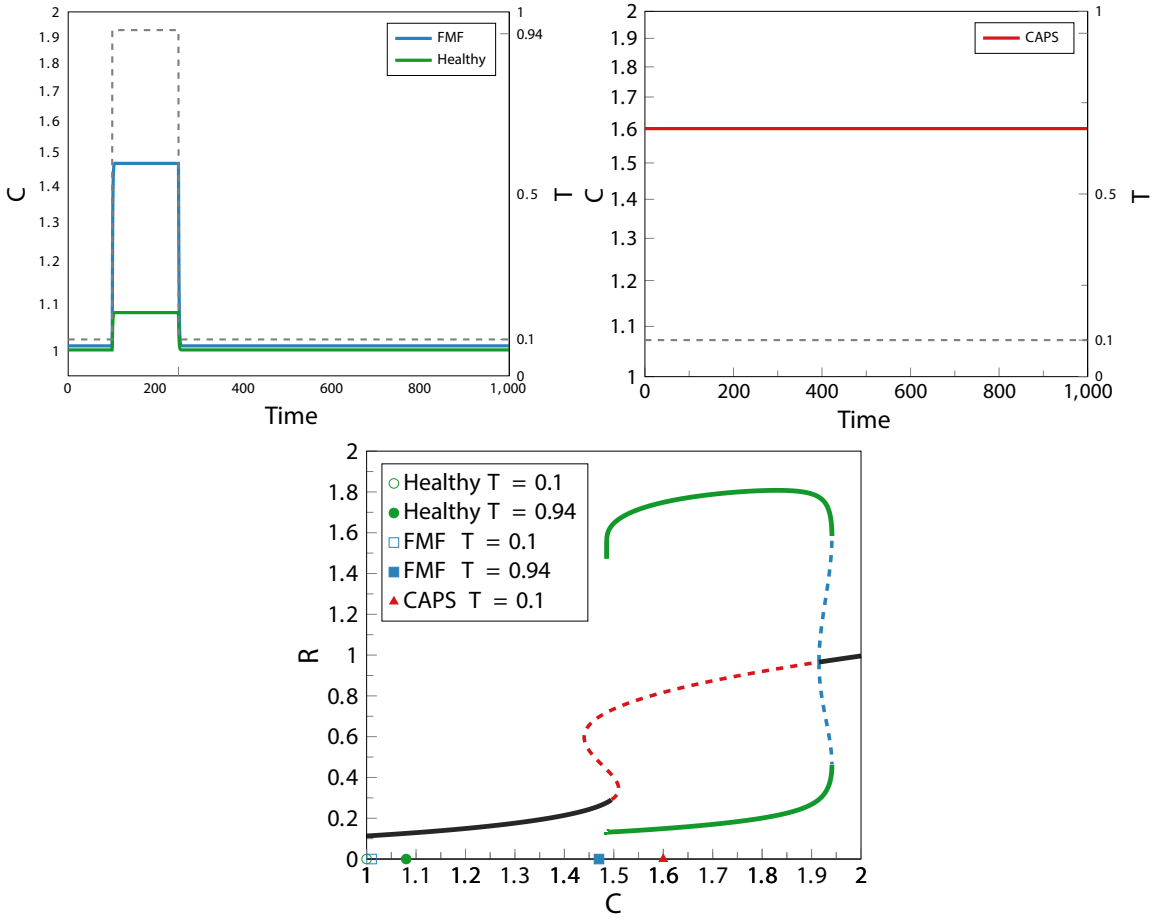


Figure 5.5: **C** Levels in *Healthy*, *FMF* and *CAPS*

As **C** is the most critical component of the model, different values correspond to different behaviors in the key constituents. In Figure 5.5, bifurcation analysis is again plotted with the markers for specific cases added. Both time simulations and bifurcation analysis justify the classification of modes. In *Healthy*, **C** levels are low independent of the **T**. In *FMF*, when **T** is low, so is **C**. High **T**, increases **C** values into excitable mode. In *CAPS*, **C** levels are always high where other key constituents are in oscillatory region.

Chapter 6

CONCLUSION AND FUTURE DIRECTIONS

In this thesis, we have presented a unified mathematical model for two of the autoinflammatory, genetically linked, innate immunity related syndromes, FMF and CAPS. These diseases are caused by the mutations in the regulatory proteins, pyrin and cryopyrin, respectively. Although the mutated regions are different, FMF and CAPS have a lot of resemblances in terms of the pathogenesis. Overproduction of IL-1β due to increase in Caspase 1 concentration is the main cause that triggers the inflammation process. That is why we tried to develop a unified model in order to capture both FMF and CAPS behaviors by adjusting the mutation parameters. Fortunately, by introducing the relevant mutations into the model, we successfully observe the clinical behaviors and elucidate the disease mechanisms. According to the simulation results, Caspase 1 level is the most critical parameter in determining the three modes that the model exhibits, *Healthy*, *FMF* and *CAPS*. In accordance with the literature, FMF is considered as trigger-dependent while CAPS is due to self dynamics of the immunity-related protein concentrations. For this reason, FMF attacks occur only when adequate trigger is initiated to the body. CAPS, on the other hand, has a periodic nature and even when there is no trigger applied, attacks occur, if not treated.

6.1 Summary

As a brief introduction, the motivation for mathematical modeling of immune diseases is given in Chapter 1 with general information on autoinflammatory and autoimmune diseases.

Chapter 2 was dedicated to the details of FMF pathogenesis with the perspective of modeling. Literature review on the possible consequences of pyrin mutation is presented in detail. Since we have followed almost a bottom-up approach in model development, molecular picture beyond FMF is

described. Normally (without mutation), pyrin binds to two important players, proCaspase 1 and Caspase 1, which prevents over-formation of cryopyrin inflammasome that produces Caspase 1. Mutated pyrin can no longer act as an effective suppressor of the inflammasome formation when an external inflammasome initiator signal (PAMP or DAMP) is present. Caspase 1 concentration, being a key for the activation of IL-1β, becomes a very critical parameter due to the fact that over-production of IL-1β starts the cascade of events, ending up in inflammation.

Chapter 3 deals with CAPS in the same manner as it was done for FMF. We have provided background information about the molecular picture beyond disease pathogenesis. Over-formation of cryopyrin inflammasome is due to a mutation in cryopyrin protein in this disease. The rest in disease progression is basically the same as in FMF, i.e., Caspase 1 levels are higher than in FMF and over-production of IL-1β is the inflammation initiator. Available literature suggests that CAPS attacks occur even when there is no external stimulus, or they have not been identified yet. We have also discussed the mechanisms of the available drugs, successes of which verify that over-production of IL-1β is the main cause of the disease symptoms in CAPS.

In Chapter 4, the unifying disease model based on enzyme kinetics has been developed. The model is in the form of five coupled nonlinear ordinary differential equations. We note that the model can be separated into two subsystems, output of one serves as an input for the other. One of the subsystems determines Caspase 1 concentration according to the presence/non-presence of the related mutations in pyrin and cryopyrin in FMF and CAPS, respectively. According to Caspase 1 concentration, final values of **R**, **I** and **A** concentrations differ in the three modes, *Healthy*, *FMF* and *CAPS*. We investigate the model by bifurcation analyses. Caspase 1 is selected as the bifurcation parameter.

Chapter 5 presents simulation results. In *Healthy* mode, even though the trigger is present, a slight increase in **C**, **R**, **I** and **A** is seen. When pyrin mutation is introduced, Caspase 1 level increases into higher values , with the presence of the trigger, when compared to *Healthy* mode. Trigger duration and magnitude is important in the determination of the final values of **R**, **I** and **A**. In *CAPS*, even though there is no trigger present, since cryopyrin is mutated, Caspase 1 levels are always higher than *Healthy* and *FMF* modes. High concentration of **C** guarantees the system to be in the

oscillatory region where maximum values of **R**, **I** and **A** are apparently the highest among the three modes.

6.2 Future Research Directions

Our work only numerically presents different behaviors when corresponding mutations for FMF and CAPS are introduced to the model. It would improve the study further if we could analyze the model analytically. In addition, it would be better if different parameter regimes verified the model. Moreover, the role of drugs might have been added to the model as a variable. Since our variables are abstract, lumped and unitless, it is hard to correlate the model to real physical quantities. It would also make more sense if the model could predict the quantitative measurements. Despite these limitations, our model qualitatively captures the overall interplay between the key constituents.

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