Mathematical Modeling of Behçet's Disease

by

Cemal Erdem

A Thesis Submitted to the
Graduate School of Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Master of Science
in
Chemical and Biological Engineering

Koc University

August 2012

Koc University

Graduate School of Sciences and Engineering

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

Cemal Erdem

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

Prof. Dr. Burak Erman (Advisor)

Assoc. Prof. Dr. Alper Demir (Co-advisor)

Prof. Dr. Yaman Arkun

Prof. Dr. Özlem Keskin

Asst. Prof. Dr. Meltem Müftüoğlu

Date: 01.08.2012

ABSTRACT

Behçet's Disease (BD) is an auto-inflammatory, immune-mediated disorder affecting skin, mucosa, eyes, blood vessels and many organs. The disease prevalence is significantly increased along the countries of the Old Silk-Road trade route. The approximate occurrence is one in a million in the USA whereas four per thousand in Turkey contract the disease. Being a center of attraction for clinical research, BD studies continue on genomic scales in some Mediterranean, Middle Eastern and Eastern Asia countries. Researchers have found out that the disease is related to an MHC protein, HLA-B51; however, the pathogenesis of BD is still a mystery. In this study, we present a mathematical model based on a dynamical systems perspective that captures especially the relapsing nature of the disease, which has no obvious correlation and differs greatly from patient to patient and time to time. We propose a disease progression mechanism and construct a model consisting of a system of Ordinary Differential Equations (ODEs), which reveals the occurrence pattern of the disease in the population. The model has three distinct modes describing the different phenotypes of people with HLA-B51, the healthy carrier, the potent patient, and the diseased. A model for the healthy population can be obtained by removing the HLA-B51 effect from our model, which is in accordance with other immune system models previously proposed and represents the normal inflammation response of the body. We present an exemplary mathematical model for BD, for the first time in the literature, that captures the actions of many cell types together with genetic, environmental, and protein effects. The proposed model provides insight into this complex immunological disease, and it may help with its treatment and prevention.

ÖZET

Behçet Hastalığı (BH) deriyi, gözleri, kan damarlarını ve daha birçok organı etkileyen ve bağışıklık sistemini de içeren otoinflamatuar bir rahatsızlıktır. Hastalık *İpek Yolu* adı verilen antik ticaret rotası üzerinde bulunan ülkelerde daha yaygındır. Amerika Birleşik Devletleri'nde milyonda bir görülen bu hastalığa Türkiye'de yaklaşık her bin kişiden dördünde rastlanmaktadır. Bazı Akdeniz, Orta Asya ve Doğu Asya ülkelerinde Behçet Hastalığı klinik araştırmaların ilgi odaklarından biri olmakla beraber, çalışmalar genetik düzeyde devam etmektedir. Araştırmacılar, hastalığın HLA-B51 adında bir majör histokompatibilite kompleksi ile ilişkili olduğunu ortaya koymuşlardır, ancak BH'nin nasıl oluştuğu halen bir sırdır. Bu çalışmada, dinamik sistemler perspektifiyle, hastalığın insandan insana ve ülkeden ülkeye değişen, kendiliğinden tekrar edebilme özelliğini yansıtan matematiksel bir model oluşturulmuştur. Burada, hastalığın oluşum mekanizmasının açıklanmasının yanı sıra bu hastalığın toplumda oluşma şablonunu ortaya koyan Türevsel Denklemlerden (TD) oluşan bir model sunulmuştur. Bu modelde HLA-B51 taşıyan insanlarda görülen fenotipleri açıklayan üç farklı tavır bulunmaktadır: sağlıklı taşıyıcılar, muhtemel hastalar ve hastalıklılar. HLA-B51 proteinin bu modeldeki etkisi çıkarıldığında sağlıklı insanlar için de bir model elde edilebilmekte, bu yeni model ise daha önce ortaya konulmuş bağışıklık sistemi modelleri ile uyumlu ve insandaki normal bağışıklık yanıtını gösterebilmektedir. Bu çalışmada, değişik bağışıklık sistemi hücrelerinin yaptıklarını ve genetik, çevresel ve protein etkilerini yansıtabilen, alanında ilk ve öncü olabilecek bir matematiksel model sunulmuştur. Sunulan model, bu karmaşık immünolojik hastalığın anlaşılmasına katkıda bulunacak ve hastalığın tedavi ve önlenmesine yardımcı olacaktır.

ACKNOWLEDGEMENTS

First of all, my advisor Prof. Dr. Burak Erman deserves gratitude more than I can ever fulfill to satisfy. His guidance has enlightened my path through the two years of this study. I am very grateful and honored to have the opportunity to work with him. My co-advisor Assoc. Prof. Dr. Alper Demir also deserves my deepest gratitude and thanks for his continuous and most valuable support and guidance during my thesis. My sincere thanks and appreciation go to Prof. Dr. Ahmet Gül. He was another precious guide and source of wisdom, without him this study would not have ever been started. I also gratefully thank the esteemed committee members Prof. Dr. Yaman Arkun, Prof. Dr. Özlem Keskin and Asst. Prof. Dr. Meltem Müftüoğlu for sparing their time and for their inestimable comments. I also would like to thank TÜBİTAK and Koç University for their financial support and for setting the ground for this study. I am more than happy to the chance meeting my former office-mates Dr. Evrim Besray Ünal, Dr. Özge Şensoy, Cahit Dalgıçdir, Beytullah Özgür, Osman Eryurt, Cemre Kocahakimoğlu, and Ece Bulut as well as my current office-mates Çiğdem Sevim Bayrak, Bahar Değirmenci, and Derya Aydın. I thank every one of them in addition to my all other friends for being there and always supportive. My deepest thanks go to my housemates Mustafa Kılıç and Teko for their endless patience and for the delicious food we shared. Last but not least, I humbly thank my parents Selma & Turgay Erdem, my sister Türkan Erdem, my brother Tolga Erdem and my prospective lifelong partner Hülya Kıraç for being a part of my life, success, and love. Their warmth presence is all I need and this work is dedicated to them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

1. INTRODUCTION

Inflammation is a part of the complex immune response to protect the body against infection or other harmful stimuli. When an organism is attacked by a pathogen or when some self-abnormalities arise, different levels of protection systems come into effect in a combined manner. Behçet's Disease (BD) is a chronic, multi-systemic, immune mediated auto-inflammatory disorder. It causes prolonged and recurrent aphtha or ulcers and tissue damage. The clinical features and manifestations of BD are well understood and agreed on [1, 2]. However, the pathogenesis or the etiology of the disease still remains a mystery.

The BD patients generally manifest recurrent oral ulcerations, recurrent genital ulcerations, eye lesions, skin lesions, and a positive pathergy test, although these manifestations may differ from country to country [3]. BD is also known as the *Old-Silk Road Disease* since its prevalence is highest in the countries along this ancient trade route [4]. For instance, Turkey, Israel and China have the highest incidence rates compared to rarity seen in American and Scandinavian countries [1, 5-7].

In studies of BD, clinical tests revealed that some bacteria, viruses, and self-antigens contribute to the development as well as onset of the disease symptoms [8-24]. More detailed genetic analysis in case-control studies is also employed. These Genome Wide Association Studies (GWAS) located hundreds of thousands of single nucleotide polymorphisms (SNPs). Among all these studies, one common result is reported so far; HLA-B51 is associated with Behçet's Disease [5, 8, 20]. The occurrence of BD in families was also investigated, showing the effect of HLA-B51 and familial inheritance in disease

prevalence [2, 4, 8]. Gül et al. reported that the overall effect of HLA-B51 in BD is only 19% [25].

In the history of disease metabolism researches, mathematical models are employed to explain how the symptoms or clinical features may manifest [26-29]. In these models, different types of approaches and equation systems are used. One such approach is to use Ordinary Differential Equations (ODEs), where selected system parts are represented as state variables [30]. To the extent of our knowledge, however, there is not any mathematical model for BD. Thus, the present study aims to fill-in this gap and to provide assistance in disease prevention. Based on both the clinical and the genetic data available, a mathematical model with a dynamical systems perspective is proposed. This model captures the recurrent nature of inflammation seen in BD patients and demonstrates the effect of HLA-B51 and different kinds of immune cells in this oscillatory, recurrent behavior. In this study, a model for the healthy population is also introduced.

In Chapter 2, a detailed literature survey is presented concerning the genetic risk factor HLA-B51, other important molecules and their signaling pathways, important cell types (Antigen Presenting Cells – APCs, innate and adaptive immune cells), and some pathogenesis maps available.

In Chapter 3, employed methods are given. Constructed maps of disease pathogenesis based on the extensive literature survey, the proposed disease progression, and the mathematical models are the elements presented in this section.

In Chapter 4, first, a comparison of one of the present models with a previous research is given. Then, the diseased model results are presented, where the modes revealed by the model and corresponding phenotypes of population are compared. The validation of the methodology is also presented.

In Chapter 5, the conclusions drawn from this study are highlighted.

Chapter 2

2. LITERATURE REVIEW

2.1. Behçet's Disease

Behçet's disease (BD) is a rare, systemic, form of vasculitis (inflammation of the blood vessels) that often includes mucous membrane ulceration and eye lesions. In BD, Mendelian inheritance is not clear. Being rather a chronic inflammation disorder, BD causes tissue damage and prolonged and recurrent aphtha or ulcers. For the diagnosis of BD, recurrent oral ulceration is a must in addition to at least two of recurrent genital ulceration, eye lesions, skin lesions, and positive pathergy test, although these manifestations may differ from country to country [1-3]. Table 1 summarizes the international consent on the criteria of BD.

Table 1 The International Criteria for Behçet's Disease [1-3]

Must have:	**Recurrent oral ulceration**	Seen at least three times a year
Plus at least two of:	**Recurrent genital ulceration**	
	Eye involvement	Eye lesions, uveitis, retinal vasculitis
	Skin lesions	
	Positive pathergy	Read by a physician at 24-48 hours

BD is also known as the *Silk Road Disease* since its prevalence is highest in the countries along this ancient trade route [4]. For instance, the highest incidence is seen in Turkey with around 420 cases per hundred thousand. In Japan, the disease is seen in 22

cases per hundred thousand. However, the disease is rarely encountered in the United States, only one case in a million [1, 5-7]. Table 2 presents some of the data for disease prevalence. The occurrence of BD in families was investigated, showing that sibling recurrence is important to indicate the genetic background of BD [2, 4, 25].

In studies of BD, firstly clinical tests were employed in order to identify what parts of the immune system are triggered. Then, some studies to determine the pathogenic triggers were carried out, both external (viruses and bacteria components) [4, 9-11], and internal (heat shock proteins) [12-15], or others [16-18]. The results of these studies revealed that some bacteria like *Streptococcus sanguis* [2, 19], *Escherichia coli* [7, 20], or viruses like HSV and Hepatitis viruses [2, 7, 21, 22], or some internal heat shock proteins (due to homology with pathogenic antigens) [23, 24], as well as others cause or at least initiate the symptoms of BD.

Table 2 The Prevalence of Behçet's Disease [6, 8]

Country	Cases per hundred thousand
Turkey	420
China	110
Korea	~30
Japan	22
Egypt	~8
USA	<1

Aside from these studies, Genome Wide Association Studies (GWAS) have been conducted to determine or validate the genetic risk factors for BD. Such single nucleotide polymorphism (SNP) studies have revealed that HLA-B51 is associated with the disease [5, 8, 20].

2.2. HLA-B51

HLA-B51, which is a Major Histocompatibility Complex (MHC) Class I molecule, takes part in the presentation of peptides to the immune cells [21]. The MHC is a large genomic region found in most vertebrates that encodes MHC proteins. MHC complexes play an important role in the immune system. HLA-B51 is a 362 amino acid long protein and complexes with β2-microglobulin, Figure 1 [31]. When an antigen is captured by an APC, one fragment of this pathogen or self-foreigner is loaded by HLA-B51 onto the cavity between its alpha helices before the whole complex translocates to the cell membrane.

The HLA-B51 contribution to BD occurrence is through various mechanisms, e.g., its connection with CD8+ T and NK cells [4, 9], and γδ-T-cells and its possible misfolding causes problems with endoplasmic reticulum stress and HSP60 associations [12]. Although there are many other candidates for disease association, most of the other genetic factors are thought to be in linkage disequilibrium with HLA-B51 [2, 5, 8, 20, 32]. The effect of HLA-B51 on disease susceptibility is around 19 % [24, 25].

In two separate genome-wide association studies (GWAS) to identify the genetic variants that underlie BD, Remmers et al. studied 311,459 single nucleotide polymorphisms (SNPs) in 1200 patients and as many controls from Turkey [5]; Mizuki et al., on the other hand, examined 320,438 SNPs in over 600 patients and over 700 controls from Japan [33]. Both groups confirmed the association of HLA-B51 and BD. Then, they exchanged their data and showed that IL-10 and IL-23R-IL-2RB2, which are critical for the immune response, are new possible susceptibility loci for BD, in both populations.

In the study of BD, the single nucleotide polymorphisms (SNPs) are studied widely thoroughout the countries on the Silk Road. The genes of interest are looked for any significant relationships between; SNPs – Target (ill) individuals – Control (healthy) individuals. The studies have revealed that HLA-B51 is significantly important in the

disease together with polymorphisms in the regions of the genes of FCN2, TRIM39, RNF39, IL10, IL23R, & IL12RB2 but not of TLR2 or TLR7. However, the exact role of any of those is still unknown.

The study of Kurata et al. shows two novel SNPs independently of HLA-B51 and HLA-A26. The results of that study suggest that RNF-39 and TRIM-39 are involved in the etiology of BD [34]. Some related SNPs are Rs9261365, near RNF-39 and Rs2074474, on exon 9 of TRIM-39. In a study of IL-12B, the most common SNP is said to be 1188A/c in 3' UTR. Although IL-12B might be associated with IL-23 signaling, it is stated that the IL-12B polymorphism is not associated with BD [35]. For an extensive list of SNPs that were shown to be associated with BD, refer to the supplemental material of [5].

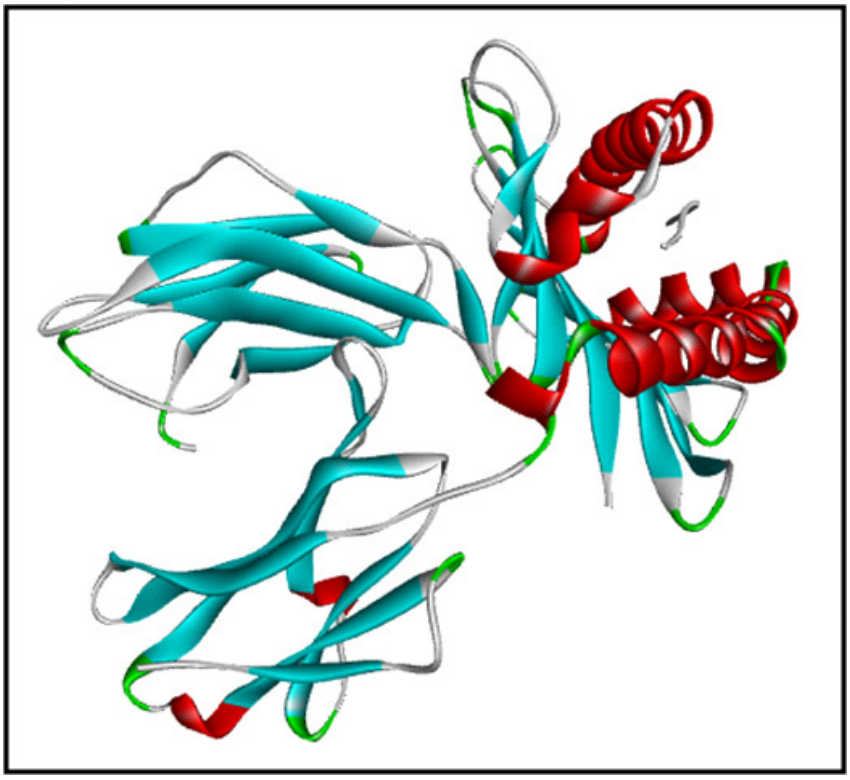


Figure 1 The structure of HLA-B51 [31]

2.3. Other molecules important in BD

Behçet's Disease is shown to be related to the specific MHC variant, HLA-B51. In addition to this protein, there are other proteins also important in the progression if not in the occurrence of BD. Some of these biological entities are: IL-12 [19, 20, 23], IFN-γ [20], ICAM-1 [36], VCAM-1 [36], IL-10 [5], IL-8 [5, 19, 20, 37], IL-18 [19, 20], TNF-α [9, 19, 20, 38], MICA [19, 21, 22, 37, 39], S100A12 [38], HSPs [23, 24, 37, 39], and other molecules [20, 37].

These secreted cytokines and others are part of a delicate mechanism that controls the overall response of the immune cells in type and magnitude to various triggers. The polymorphisms in these molecules cause or may be causing alterations in the immune response. For instance, IL-10 is related to the suppression of inflammation for especially the Th1 type response. In a Th1 response, the major cytokines are IFN-γ and IL-12 [40] and the T cells become cytotoxic, killing cells upon contacting them. IL-23R is associated with the neutrophilic response and TNF-α causes a more innate immune response. MICA is related to innate immune response activation such that MICA polymorphisms accompanying the strong linkage disequilibrium with HLA-B51 may play an important role in the development of BD via increased killer activities of T-cells, and NK cells [32]. IFN-γ drives Th1 cell proliferation whereas it inhibits Th2 cell proliferation. In a Th2 response, the T cells become B cells and differentiate into either memory cells or plasma cells, producing antibodies. IL-10 and IL-4 inhibit Th1 production where IL-4 enhances Th2 cell production. In Th1 cells, IFN-γ and TNF-α cause enhanced production of IL-12 and IL-18 (may act on itself as well as those from neutrophils). IL-8/17/18 by APCs and T-cells cause more IL-12/18 production from neutrophils.

Next, a number of proteins that are related to BD are discussed in detail.

1.3.1. MICA

It is apparent that MHC molecules play an important role in the immune system and autoimmunity. They present antigen fragments to immune cells and initiate an immune response. If there is malfunctioning in a step of this process or in a structure on the path, an undesired outcome occurs: either a no response or a chronic flare/remission cycle. One such molecule of interest is MICA (MHC class I polypeptide-related sequence A). It is a stress inducible antigen, mainly expressed by ENDs (endothelial cells) and fibroblasts. MICA interacts with a receptor (NKG2D) found mostly on natural killer (NK) cells and some T cells (CD8+ T cells and γδ T cells). This interaction leads to the activation of these cells and cause different levels of immune response [32], which is primarily related to the innate immune system. This response is enhanced in Th1 type mediums, present in the BD cases [39].

Increased expressions of MICA gene as well as overlapping of this increased expression sites and BD lesions are also reported [32]. The endothelial injury in BD can be a result of anti-MICA responses [39]. NKG2D activated immune cells are shown to increase in BD patients, also. Although the MICA association with BD is studied widely, there are no certain findings. However, some alleles of MICA are reported to be increased in active BD patients [41]. It is clear that polymorphisms in MICA gene causes increased actions of killer cells. Thus, together with HLA-B51 linkage disequilibrium, MICA plays a role in BD.

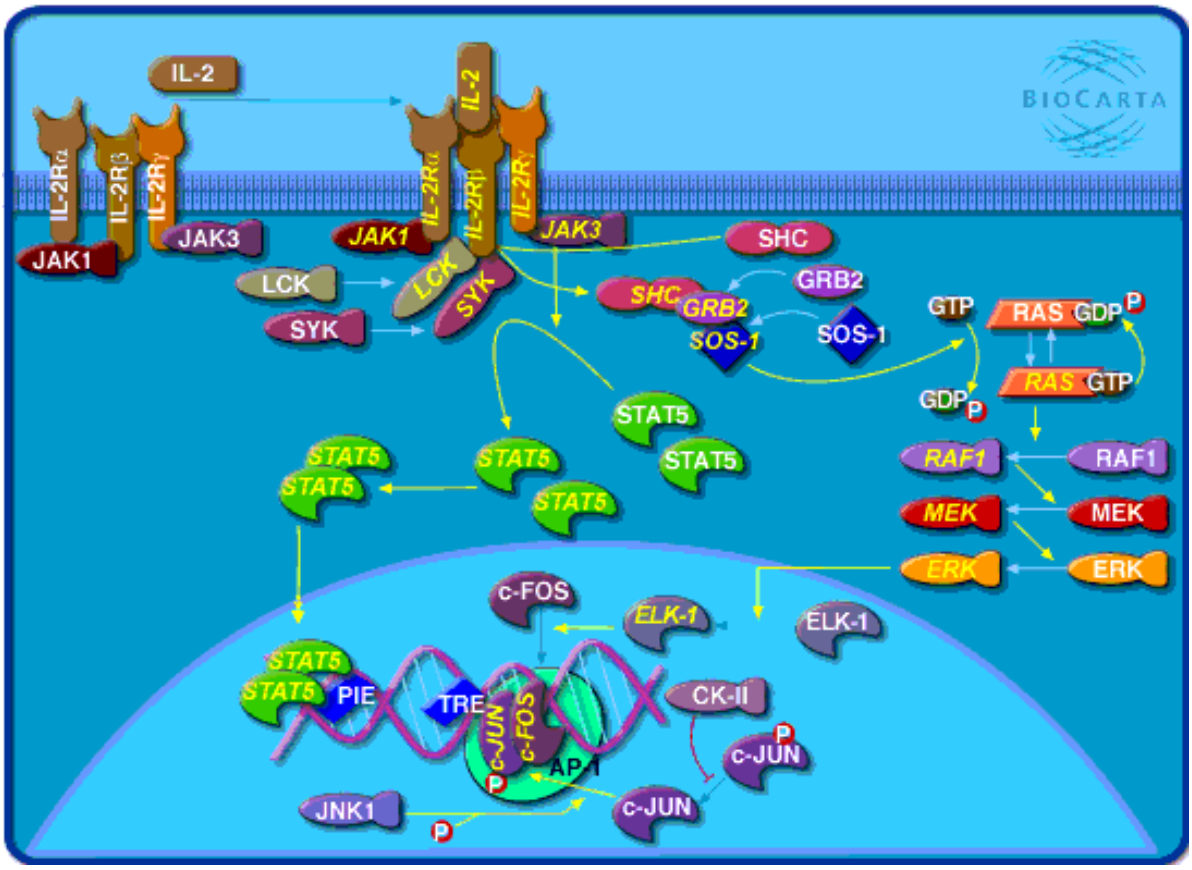


Figure 2 The IL-2 signaling pathway

2.3.2. Interleukin 2

The activated T-cell pathways result in IL-2 production and release. Then, the IL-2 works as an autocrine, leading to cell survival. The signaling pathway of IL-2 is depicted in Figure 2. As illustrated in this figure, the IL-2 receptor, IL2R, is a trimer composed of α-β-γ subunits. The Janus Kinases 1 and 3 (JAK1 and JAK3), which are bound to subunits of the IL2R, are activated by getting phosphorylated upon ligation of IL-2 with its receptor. Then, these two kinases phosphorylate Signal transducer and activator of transcription-5 (STAT5), Spleen tyrosine kinase (SYK), and lymphocyte-specific protein tyrosine kinase (LCK). SYK and LCK induce expression of two genes, namely c-Myc and c-Fos,

respectively. Moreover, by means of two other paths, more transcription factors are induced so as the IL-2 and IL2R-α chain production. One of these paths is the Phosphatidylinositol 3-kinase (PI3K) path from which the Nuclear factor-kappa B (NF-kB) is induced and v-Akt murine thymoma viral oncogene homolog (AKT) signaling is invoked. The suppression of IL-2 signaling is maintained by IL-2 induced Suppressor of cytokine signaling 1 (SOCS1) action and Transforming growth factor-beta (TGF-β) induced SMAD 3 and 4 binding to the negative regulator region of IL-2 gene.

2.3.3. Tumor Necrosis Factor – alpha

Tumor necrosis factor – alpha (TNF-α) acts in phosphorylation of intracellular proteins and in neutrophil priming [21]. The TNF-α signaling is shown in the Figure 3. In that figure it is shown that TNF-α signaling is enhanced by the receptor TNFR1 (TNF receptor 1). When TNF-α binds to its receptor, TRADD (Tumor necrosis factor receptor type 1-associated Death domain) binds to TNFR1. Then, three other molecules are bounded to this death domain; RIPK1, FADD, and TRAF2 (Receptor-interacting serine/threonine-protein kinase 1, Fas-Associated protein with Death Domain, and TNF receptor-associated factor 2). RIPK1 and FADD act together by invoking the caspase cascade leading to the cell apoptosis. On the other hand, RIPK1 and TRAF2 act together to NF-kB induction thus cell survival. TRAF2 binds to and forms a complex with c-IAP1 (Inhibitor of apoptosis 1) to inhibit caspase 8 cleavage and thus its activation. Moreover, TRAF2 activates JNK (c-Jun N-terminal kinase) cascade where mitochondrial SMAC protein is released and binds to c-IAPs to release the inhibitory effects of those molecules on caspases. The inhibitors of TNF-α signaling are ARTS-1 (Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator); by decreasing TNFR1 number in the membrane, BRE (BRCA1-A complex subunit); by inhibiting the NF-kB cascade, and SODD (Silencer of death domain); by acting onto the receptor when the ligand is absent and it dissociates upon ligation. In addition to the TNF-α, it is also shown that TNF-β1 and TNF-β2 (TNF-beta

1/2) are seen to be produced more by the monocytes in BD [19].

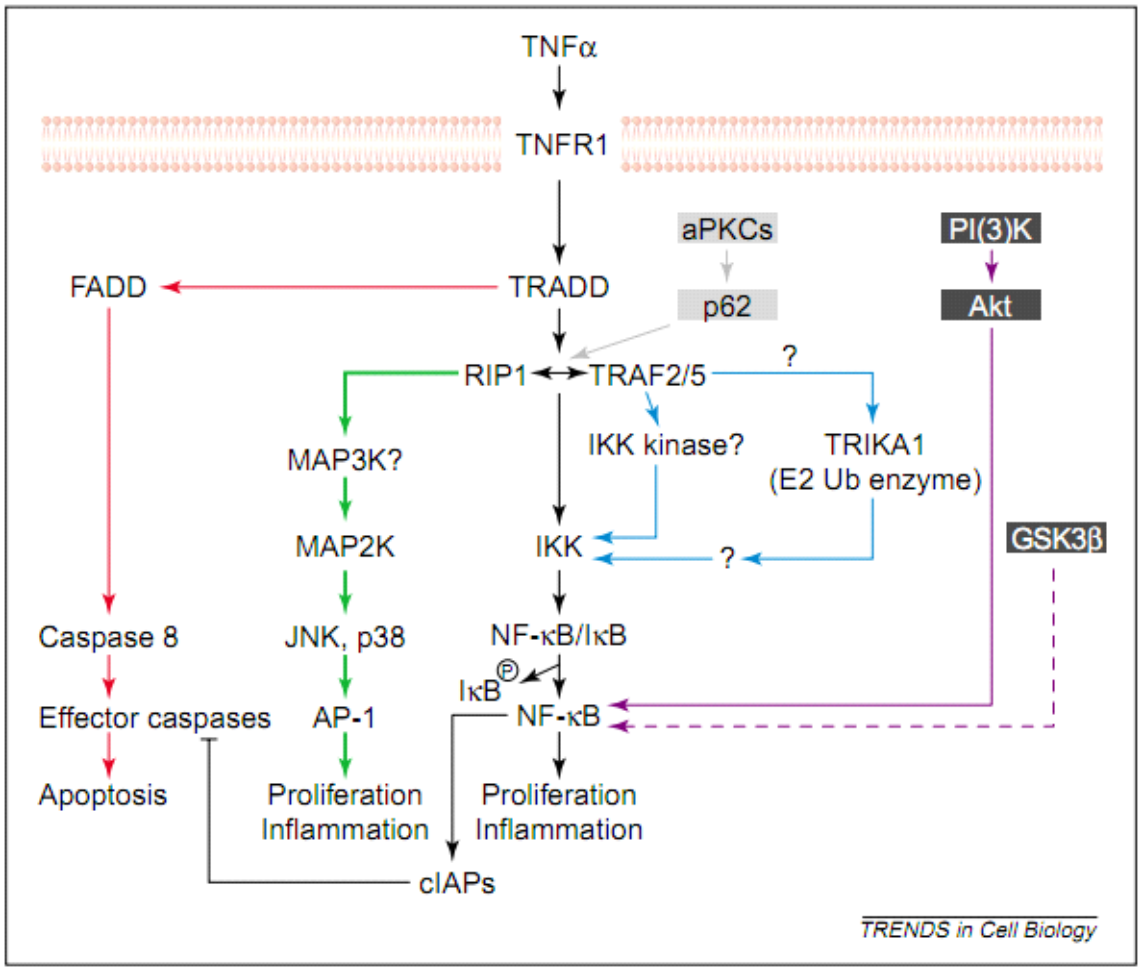


Figure 3 The TNF-a signaling map [42]

2.3.4. Interferon – gamma

One of the cytokines important in the T-cell response is the Interferon-gamma, IFN-γ, which indeed drives the Th1 (T-helper 1) type cell production as well as inhibiting Th2 (T-helper 2) type proliferation. IFN-γ is produced by activated T cells and natural killer (NK) cells. Figure 4 summarizes the signaling cascades invoked by IFN-γ.

When IFN-γ binds to its receptor, Janus kinases 1 and 2 (JAK1 and JAK2) bind to IFNGR (Interferon-gamma receptor) and trans- phosphorylate each other and thus the receptor complex becomes activated. The phosphorylated and receptor bound JAKs phosphorylate the receptor. Signal transducer and activator of transcription 1 (STAT1) binds to that phosphorylated site of the receptor. JAKs then phosphorylate STAT1, which dimerize and travels into the nucleus where it regulates some genes. The STAT1 down-regulates c-Myc leading to cell cycle progression disruption. On the other hand, STAT1 also induces the expressions of both IFN-γ and IL-12R-β2, leading to Th-1 type polarization.

Another path invoked by the IFN-γ results in the expression of Intercellular adhesion molecule 1 (ICAM-1). IFN-γ leads to Phospholipase C-gamma 2 (PLC-γ2) activation with phosphorylation by the JAKs. Subsequent events including Diacylglycerol (DAG) production leads to ICAM-1 production which is important in infiltration of neutrophils and others to tissues in immune response against infection.

IFN-γ also induces expression of Interferon regulatory factor 1 (IRF-1), which in return induces the expression of the inhibitor of the IFN-γ, the Suppressor of cytokine signaling 1 (SOCS1). SOCS1 binds and inhibits the JAKs bound to IFNGR.

2.3.5. Interleukin 10

Another important factor, known to have an effect in disease progression [Mizuki 2007], is Interleukin 10 (IL-10). IL-10 is a cytokine with anti-inflammatory properties: it represses the expression of inflammatory cytokines; TNF-α, IL-6, and IL-1β in macrophages. Functional IL-10 receptor complex is a tetramer consisting of two identical ligand-binding subunits (IL10Rα) and two identical accessory signaling subunits (IL10Rβ) [43, 44].

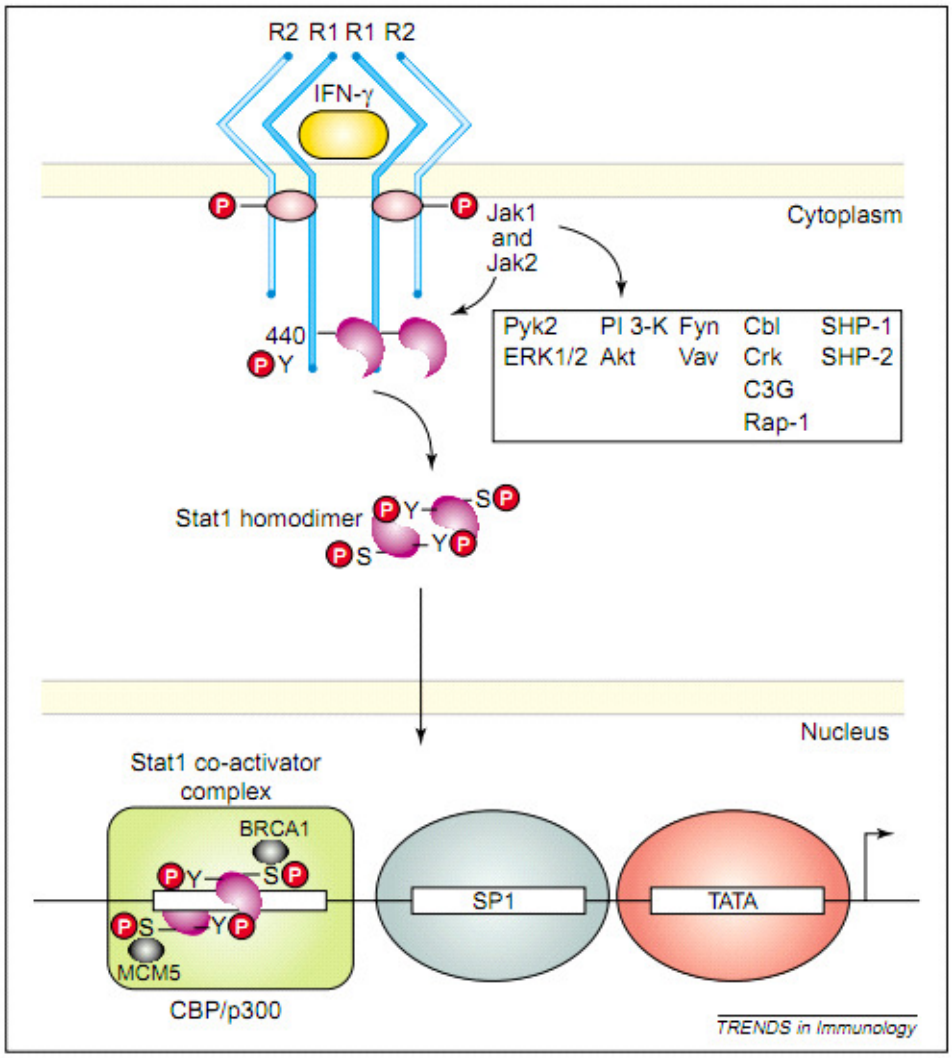


Figure 4 The IFN-g signaling scheme [45]

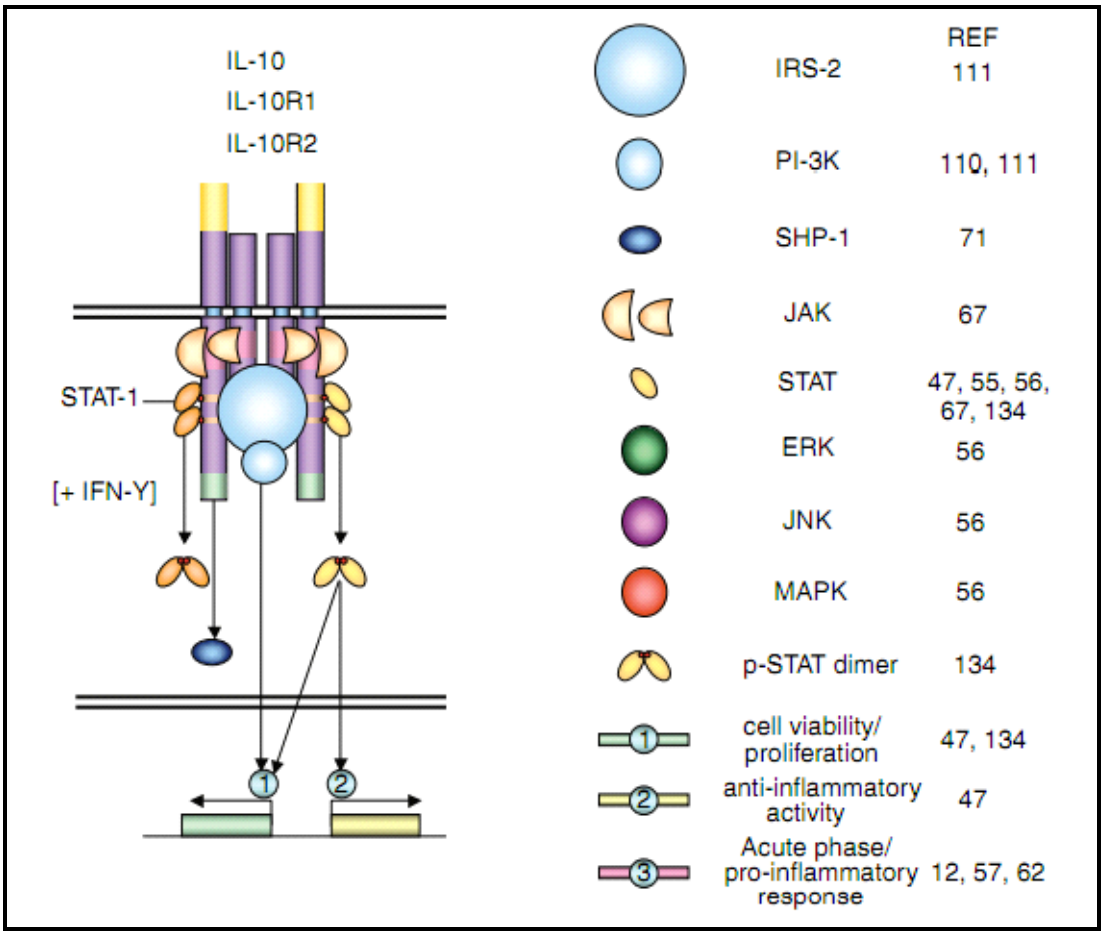


Figure 5 The IL-10 signaling pathway, adopted from [43]

Upon binding of IL-10 to its receptor, JAK1 and Tyrosine kinase 2 (TYK2) also bind to IL-10 receptor (IL10R) and they got phosphorylated, Figure 5. Then these two kinases phosphorylate some tyrosine residues of IL-10R. Phosphorylated IL10R provides sites for STAT3 docking, where it also gets phosphorylated by JAK1 and TYK2. After activation two STAT3 dimerize and goes into the nucleus to induce corresponding genes to alter expressions of TNF-α, SOCS3, Bcl-2 (B-cell lymphoma 2), and some others [46]. TNF-α is down-regulated. SOCS3 inhibits TNF-α and IL-1β expressions by negatively regulating the STAT3 activation. Bcl-2, on the other hand, inhibits cell cycle progression

leading to apoptosis. On the contrary to this Bcl induced apoptosis, JAK1 phosphorylates Insulin receptor substrate 2 (IRS-2), which causes AKT pathway activation leading to cell survival actions [47, 48].

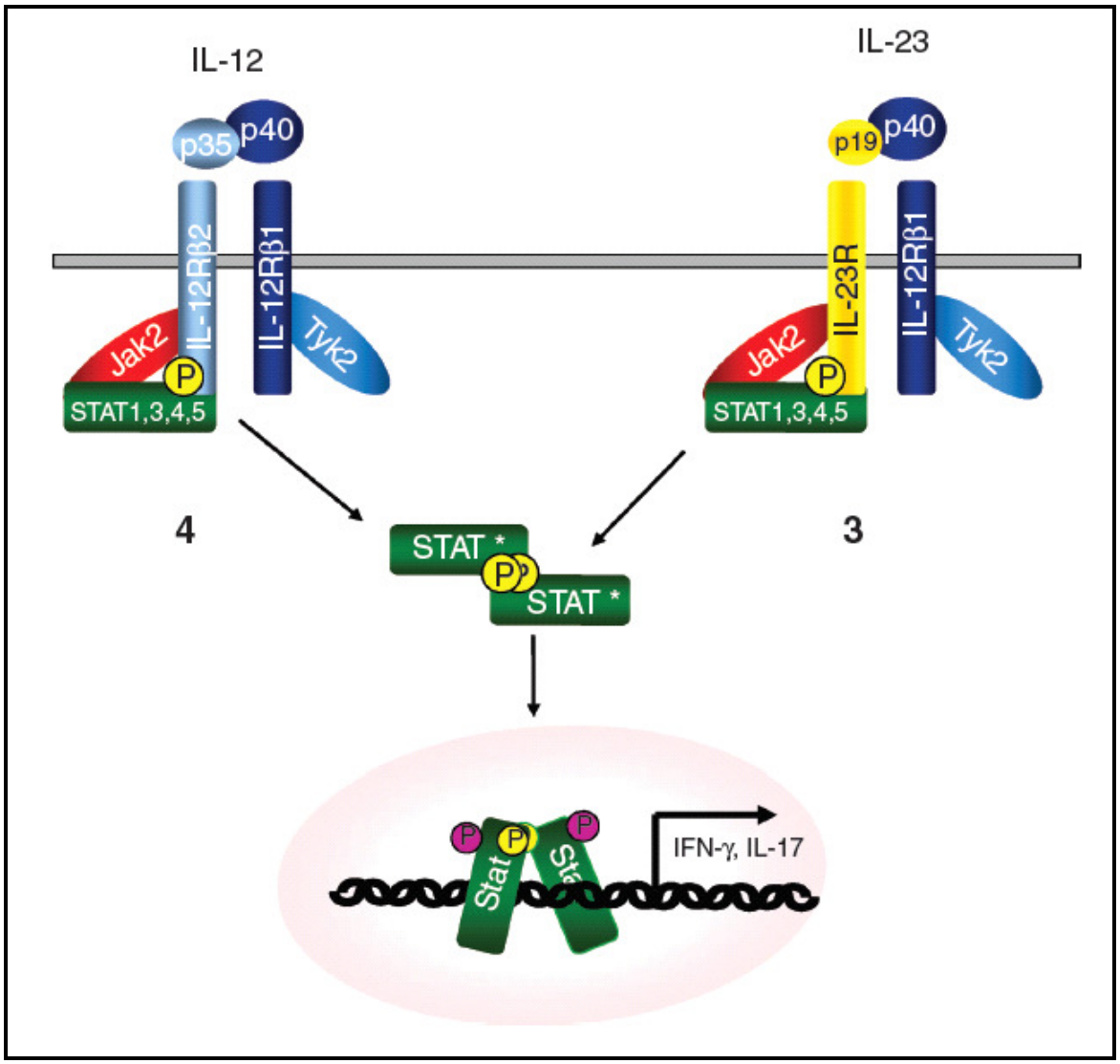


Figure 6 The IL-12 signaling pathway [49]

2.3.6. Interleukin 12

Interleukin 12 (IL-12) is another Th1 type cytokine, which alters cell dynamics with the actions of STATs it activated. It causes proliferation and differentiation of native T cells into Th-1 cells [49]. Binding of IL-12 to its complex receptor (IL12R) causes JAK2 and TYK2 be recruited also to the receptor. Activated JAK2 phosphorylates STAT3 and STAT4, which translocate to the nucleus and cause IFN-γ expression to increase. STAT4 also increases expressions of IL-18R1 (Interleukin 18 receptor 1), IL-12β2, IRF1 (Interferon regulatory factor 1), and IL2Rα [50-52]. The details of IL-12 pathway are given in Figure 6.

IL-12 also induces STAT5 activation by JAK2 phosphorylation. STAT5 promotes proliferation of the cell, but STAT5α (part of STAT5) results in SOCS3 production, which binds to and inhibits the activity of IL-12β2 [53, 54].

2.3.7. Interleukin 1

Interleukin-1 (IL-1) is a proinflammatory cytokine. It has two main types; IL-1α and IL-1β. There is also a protein called Interleukin 1 receptor (IL1R) accessory protein (IL1RaP) that aids in signaling [55-57]. The details of IL-1 signaling are depicted in Figure 7.

The protein MyD88 (Myeloid differentiation primary response gene 88) binds to IL-1R complexed with its ligand and then it activates IRAK1 and IRAK4 (Interleukin-1 receptor-associated kinases 1 and 4). Indeed, IRAK4 activates IRAK1 by phosphorylation. IRAK1 leads to activation of NF-kB and Activator protein 1 (AP-1) pathways through action of an ubiquitin ligase, namely TNF receptor-associated factor 6 (TRAF6), and subsequent downstream proteins [58, 59].

TRAF6 causes destruction of the inhibitor of NF-kB, I-kB and thus enhance NF-kB activity. As a result, the expressions of IRF1, iNOS, TNF-α, IL-6, and IL-8 increase [60-

62]. In resting cells, a protein called Toll interacting protein (TOLLIP) complexes with IRAK1 and inhibits its activation by halting its phosphorylation.

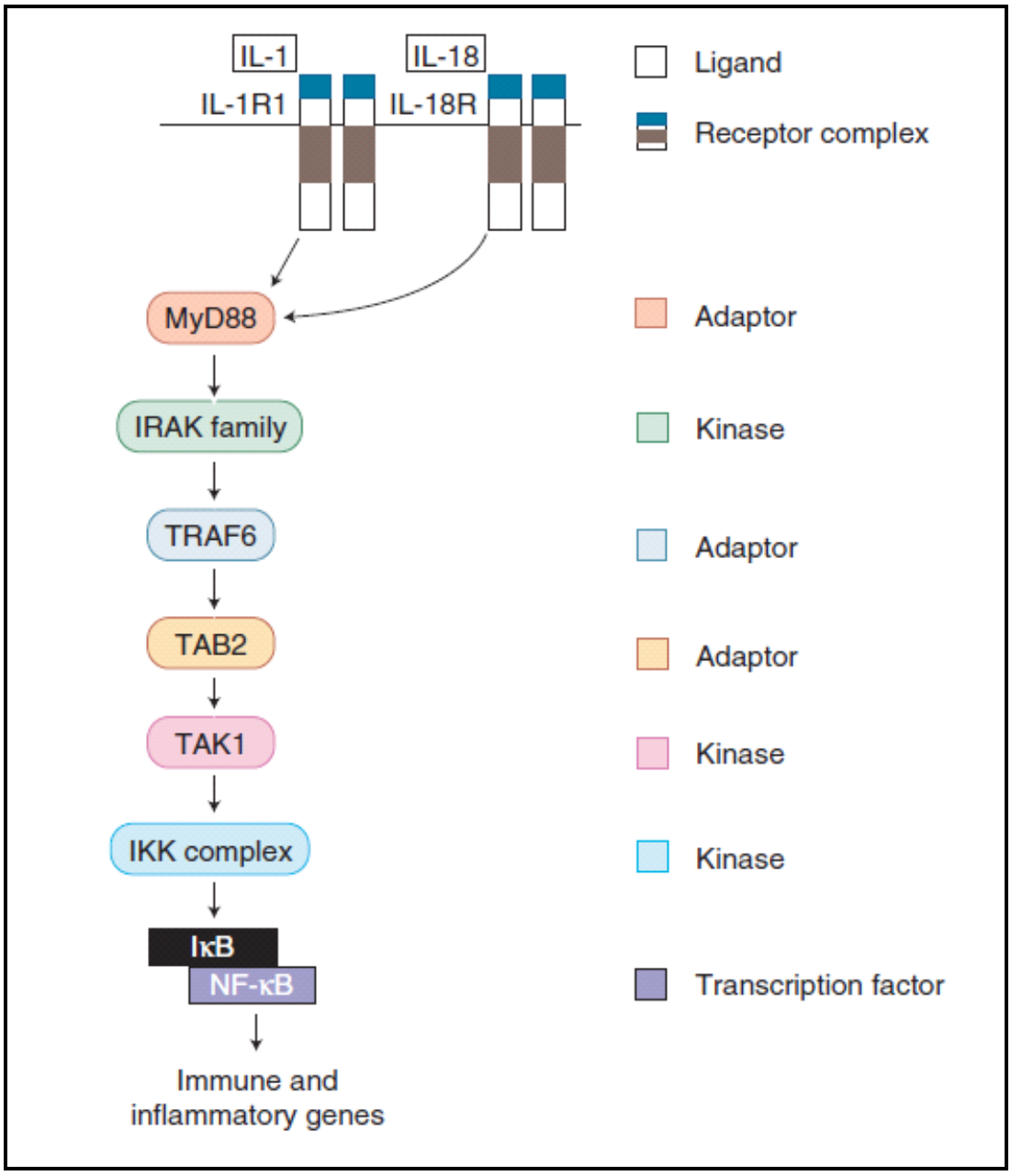


Figure 7 The IL-1 signaling pathway, adopted from [58]

2.3.8. Toll-like receptors

Toll-like receptors (TLRs) are pathogen recognition receptors and they function in identification of foreign materials. Although it was reported that Th1 type immune response is a result of activation of TLR-7, there reported no relationship between TLR-7 gene polymorphisms and BD susceptibility [63]. Another study on TLR-2 revealed five possible SNPs as contributors to BD development. However, it is concluded that TLR-2 polymorphisms also do not contribute to the development of BD. Moreover, a study in Turkish patients by Bacanli et al. stated that a TLR-2 polymorphism showed no association with BD [23]. However, the TLR-2 ligands, HSP and LPS may act as risk factors where TLR2 activation yields NF-kB action and increases TNF expression. Finally, the study in [23] shows that a polymorphism in TLR-4, the receptor recognizing HSP and LPS, is significantly associated with the risk of BD.

2.3.9. Heat Shock Proteins

Stress is the cause of heat shock protein (HSP) secretion where HSP-47 is called α-enolase. Its expression on the cell surface results in auto-antibody formation and immune complex formation resulting in END injury. The binding of HSP-70 to TLRs (TLR 2/4 with CD14 as the cofactor of binding) on APCs cause pro-inflammatory cytokine releases. HSP-60 results in rapid inflammation and Th1 polarization [37]. Moreover, certain HSP60 peptides are recognized by γδ T cells, which may be important in BD pathogenesis and where these cells produce IFN-γ and TNF-α [24]. The HSP60 is over-expressed in the oral mucosa and skin lesions of patients with BD [39].

In addition to these, the study by [20] says that four peptides included within mycobacterial 65-kDa HSP are responsible for the proliferation of γδ T cells in patients with BD. These peptides are reported to be demonstrating considerable likelihood with those of human 60-kDa mitochondrial HSP. The study also states that cross-reaction

between the microbial HSP and human HSP might be the basis for the relation of infection with autoimmunity.

Hypersensitivity response to certain *Streptococcus sanguis* antigens in the skin and sanguineous monocytes in BD patients is also present. These bacterial antigens are similar to heat shock proteins. S.anguis and especially HSP-60/65 kDa activate T cells with gamma and delta chains in BD patients but not in controls [19]. HSPs are upregulated in lesions of BD and they are higher in BD patients. Nara et al. surmised that IL-12 produced by TLR-2 expressing cells and TLR-2/HSP-60 interaction contributed to Th-1 dominant immune responses in intestinal BD [23].

2.3.10. Others

IL-8 is an attractant for neutrophils, besides leukocytes, [5, 37] through the CXCR1/2 receptors present on these cells. IL-8 also activates ENDs so that monocytes bind more tightly to the vascular ENDs [5]. E-selectin is also seen to be activated in ENDs [19]. The IL-17 is produced primarily by CD4+ and CD8+ T cells [20].

A calcium binding protein, S100A12, is also secreted during inflammation by either corresponding tissues or neutrophils in the bloodstream [38]. This protein is significantly increased during active BD (aBD) compared to the controls. Binding of S100A12 to a cell surface molecule results in a signal cascade activation yielding NF-kB and corresponding cytokine release, like TNF-A. In return, TNF-A, which is elevated in BD patients, stimulates S100A12 secretion together with NF-kB activation. As a result, the TNF-A and S100A12 are “a positive inflammatory feedback loop” present in BD pathogenesis [38]. S100A12 also dictates more expression of ICAM-1 and VCAM-1 by ENDs.

In [21], a ninemer epitope is labeled as “Behcetogenic”. Moreover, oestrogen decreases E-selectin and IL-6 gene expressions together with the superoxide generation from the neutrophils. These effects of oestrogen, according to [21], are a possible reasoning

that the clinical manifestations of BD in women are milder than in men.

The protein, FCN-2 activates immune cells to produce TNF-alpha, IL-6, and IL-8. A GWAS study among SNPs of FCN-2 gene revealed that there were no significant differences in FCN-2 gene SNPs between BD patients and controls. On the other hand, significant differences in allele frequencies of FCN-2 gene SNPs among HLA-B51 positive BD patients may reveal possible FCN contribution to innate immunity of BD patients [64].

A study on MBL gene stated that three polymorphic sites in exon 1 of MBL gene may be related to BD. Finally, it is said that mutation at codon 54 may lead to susceptibility to bacterial infections and affect the innate immune system in BD [65].

2.4. Cells

The disease is related to the actions of Antigen Presenting Cells (APCs) [20], T-cells [13, 36, 37, 39, 66], and neutrophils [67]. The hyperactivity of these cells results in tissue damage and inflammation. The connections among these cell types and their up/down regulation cycles are regulated by the cytokines and chemokines produced and released by the cells. When an antigen enters the body, it is captured by the APCs. Then, the antigen is fragmentized into smaller pieces after which the small peptide fragment is bound to MHC polypeptide in the endoplasmic reticulum (ER) compartment. The antigen bound MHC complex translocates to the cell membrane for the display of the antigen to immune cells, Figure 8. These activated immune cells, then, can proliferate and kill the infected cells. The interaction between T cell receptor (TCR) and antigen presenting MHC complex is illustrated in Figure 9. The T-cell and APC interaction mediates signaling in the T-cell for proliferation. T-cell proliferation and the release of IL-2 from these cells induce other neighboring T-cells to proliferate also. This activation of T-cells then results in cytokine release and neutrophil activation as well as Th1 type immune response to progress.

In BD, the keratinocytes (KERs) and endothelial cells (ENDs) [2, 18, 36, 68] are the first in line to contact the triggers and release proinflammatory cytokines. These cells are basic protection against infections, and with the help of secreted molecules they cause immune cells (neutrophils [2, 20, 38, 67, 69], monocytes [70, 71], and NK cells [13, 66, 72, 73]) to accumulate at the inflammation sites.

Like other APCs, macrophages induce an immune response, in favor of Th1 type, where IFN-γ plays a critical role in activation of macrophages taking part in regulation of antigen representation [74]. Together with CD40L, IFN-γ acts in activation of macrophages and monocytes to produce monokines causing APC stimulation and MHC Class II molecules expression and, thus, finally resulting in T cell activation [19].

After the APCs are loaded with the antigens, the innate immune cells come into action while causing more cytokine release so that the adaptive immune response is also triggered. During the pro-inflammatory cytokine production by the innate immune cells, monocytes produce IL-1, IL-6, IL-8, and TNF [19, 70, 71, 75], and neutrophils primarily release superoxides and many other cytokines [19, 20]. In BD patients, the presence of an increased superoxide production by neutrophils is shown to be in accordance with the presence of HLA-B51 [76].

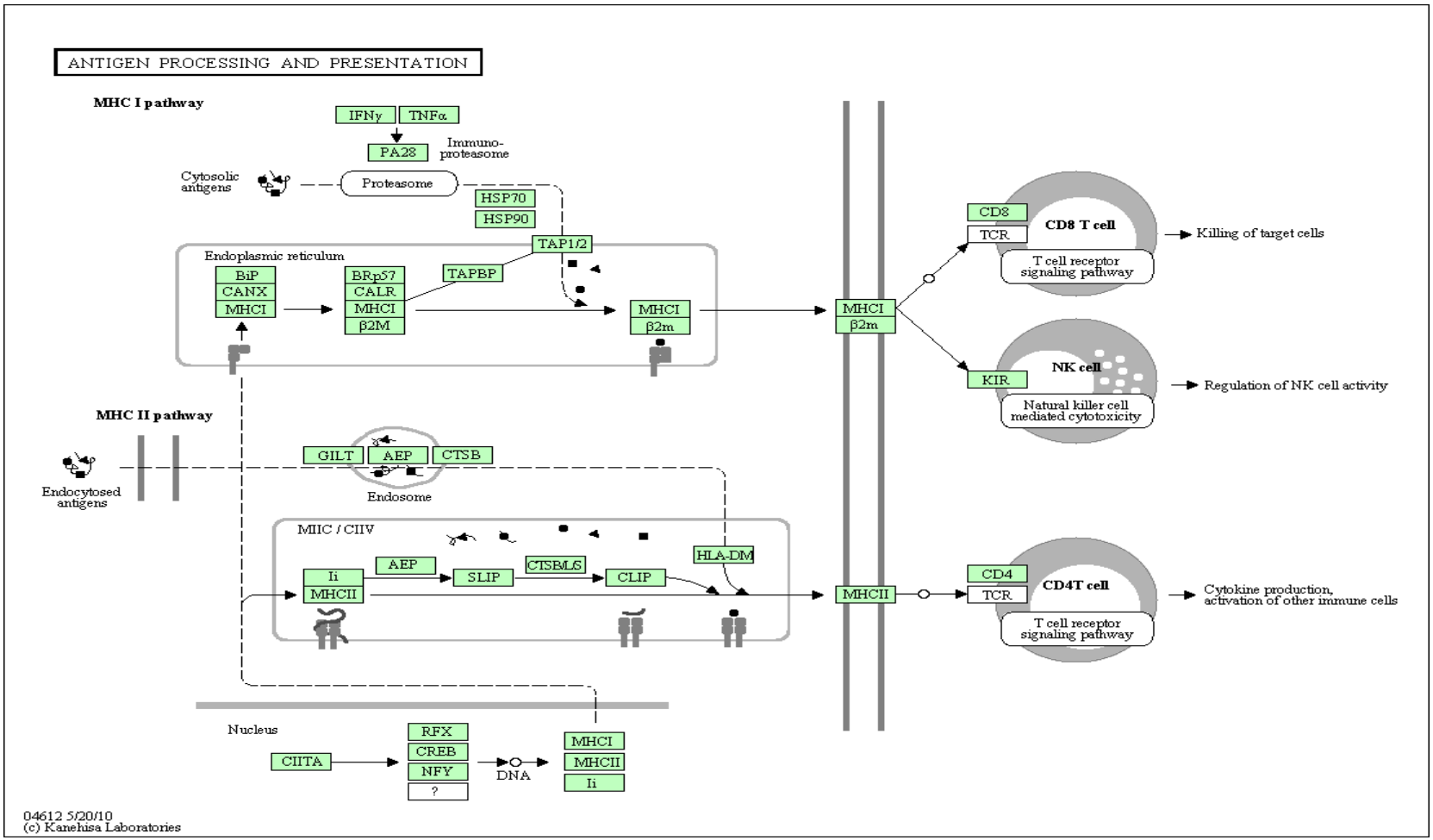


Figure 8 The MHC-I and MHC-II type antigen presentation by APCs [77]

In inactive-BD (iBD), the NK cells carry out some duties including lowering of IL-12R-β2 and perforin production in contrast to higher IL-13 numbers. Moreover, IL-12 induced STAT4 phosphorylation is also cracked [72]. Thus, the Th1 type response is suppressed by lower IL-12 processing and thus lowers IFN-γ production. The IL-12 present in the Th1 environment activates NK cells [45]. During iBD, NK and T cells, which produce IL-4 and IFN-γ in early stages of inflammation, are claimed to be deficient in IFN-γ production [72].

The neutrophils are kept in primed condition as a result of pro-inflammatory cytokines [67]. The cause of hyperactivity of neutrophils, however, is the result of the presence of IL-17, IFN-γ, IL-8, and TNF-α [20]. The primed neutrophils express CD40 and CD83 on their surfaces and produce IL-1, IL1RA, MIP, IL-12, and IL-18 [20] as well as secreting more ROS (reactive oxygen species) [19]. In BD patients, the presence of an increased superoxide production by neutrophils is due to the presence of HLA-B51 [76].

As illustrated by Figure 8, the peptide (antigen fragment) bound MHC polypeptide on the APCs binds to T-cell receptor (TCR)-CD3 complex. Then, the glycoprotein CD4 on the T-cell also binds to antigen-MHC complex at some other part, stabilizing the prior interaction [78]. Another signal, a *costimulatory* signal for further progress of signal continuation in the T-cells is also needed. The second signal mentioned is performed by the action of T-cell membrane element CD28, where it binds to CD80 or CD86 found on APCs, and the mentioned signal transduction starts in T-cells. On the other hand, CD152 or CTLA-4, Cytotoxic T-Lymphocyte Antigen 4, can also bind to CD80 or CD86 on APCs, which is the T-cell activation inhibitor.

The TCR ligation leads to subsequent ZAP70, Zeta-chain-associated protein kinase 70 (it is a tyrosine kinase) activation. Activated ZAP-70 phosphorylates adaptor protein Linker of Activated T cells, LAT which in turn turns on PLC-g1 (Phospholipase C-gamma 1) activation upon binding to it. The PLC-g1 activation finally yields NF-kB pathway activation together with Ca2+ release and subsequent nuclear factor actions promoting

immune response genes' expressions. Moreover, the AP-1 transcription factors are activated also by the action of phosphorylated LAT binding to Growth factor receptor-bound protein 2, GRB2. The first signal effect, which is summarized here, is given in Figure 9.

The receptor CD28 activation enhances the signal started by TCR activation. In response to ligand activation, CD28 binds to phosphatidylinositol kinase 1 (PIK3R1A), GRB2, and some others. The whole picture of second signal is represented in Figure 9. CD28 binds to SLP-76 with the help of GRB2 and GRAP2. TCR causes ZAP-70 activation, and phosphorylation of LAT and SLP-76. LAT then is able to bind to the SLP-76 after which SLP-76 recruits VAV-1 (Proto-oncogene vav) which finally induces c-Jun (a transcription factor) activity, through MEKs/JNK pathway, thus cell proliferation. Moreover, the CD28 bound ITK induces PLC-g1 path to be activated that result in transcription factor activity of NF-ATs, Nuclear factor of activated T-cells. The ITK also activates Akt signaling, which acts to inhibit NF-AT nuclear translocation.

In the secondary response of adaptive immune cells, the type of molecules in the vicinity dictate the type of adaptive response, where, in BD, a Th1 type is favored [4, 8, 19, 20, 23, 37, 74] not a Th2 response. Many researchers identified a reduced CD4+/CD8+ T cell ratio resulting from both a decrease in CD4+ T cells and an associated increase in CD8+ T cells [4]. When the body is infected, the naïve T cells complexed with an APC start to proliferate and release IL-2 [20]. This cytokine then acts as an autocrine to further maturate the source cells together with the other cells around the secreting cell. Then, with the attack of the adaptive immune system, the T-cells proliferate.

The naïve T cell complexed with an APC starts to proliferate and release IL-2. This cytokine then acts as an autocrine to further maturate the source cells together with the other cells around the secreting cell. Then the T cell has two possible paths to take, either Th1 or Th2. In Th1 response, the major cytokines are IFN-G and IL-12 and the T cells become cytotoxic, killing cells upon contacting them. In Th2 response, however, the T cells

become B cells and differentiate into either memory cells or plasma cells, producing antibodies for killing.

Various abnormalities of T cells have been described in patients with BD. Many researchers identified a reduced CD4+/CD8+ T cell ratio resulting from both a decrease in CD4+ T cells and an associated increase in CD8+ T cells. The elevated T cells were shown to express activation markers, such as CD25, CD69, and CD29 and produce inflammatory cytokines including IFN-γ, TNF-α, and IL-8 [20]. Increased chemotaxis, phagocytosis, superoxide generation and myeloperoxidase expression, as well as enhanced expression of CD11a, CD10, and CD14 on the cell surface have been reported in the neutrophils of BD patients. Moreover, reduced CXCR2 chemokine receptor expression on the surface of the synovial neutrophils was shown in BD patients as well as MALP-404, lipoprotein of mycoplasma fermantase, was detected in the sera of BD patients. Due to some homological peptides between MALP-404 and HLAB51, it is said to be possible that infection with mycoplasma might be involved in the pathogenesis of BD [20]. Moreover, it is included that a single-nucleotide polymorphism located at position -607 of IL-18 gene promotor region in BD is also characterized.

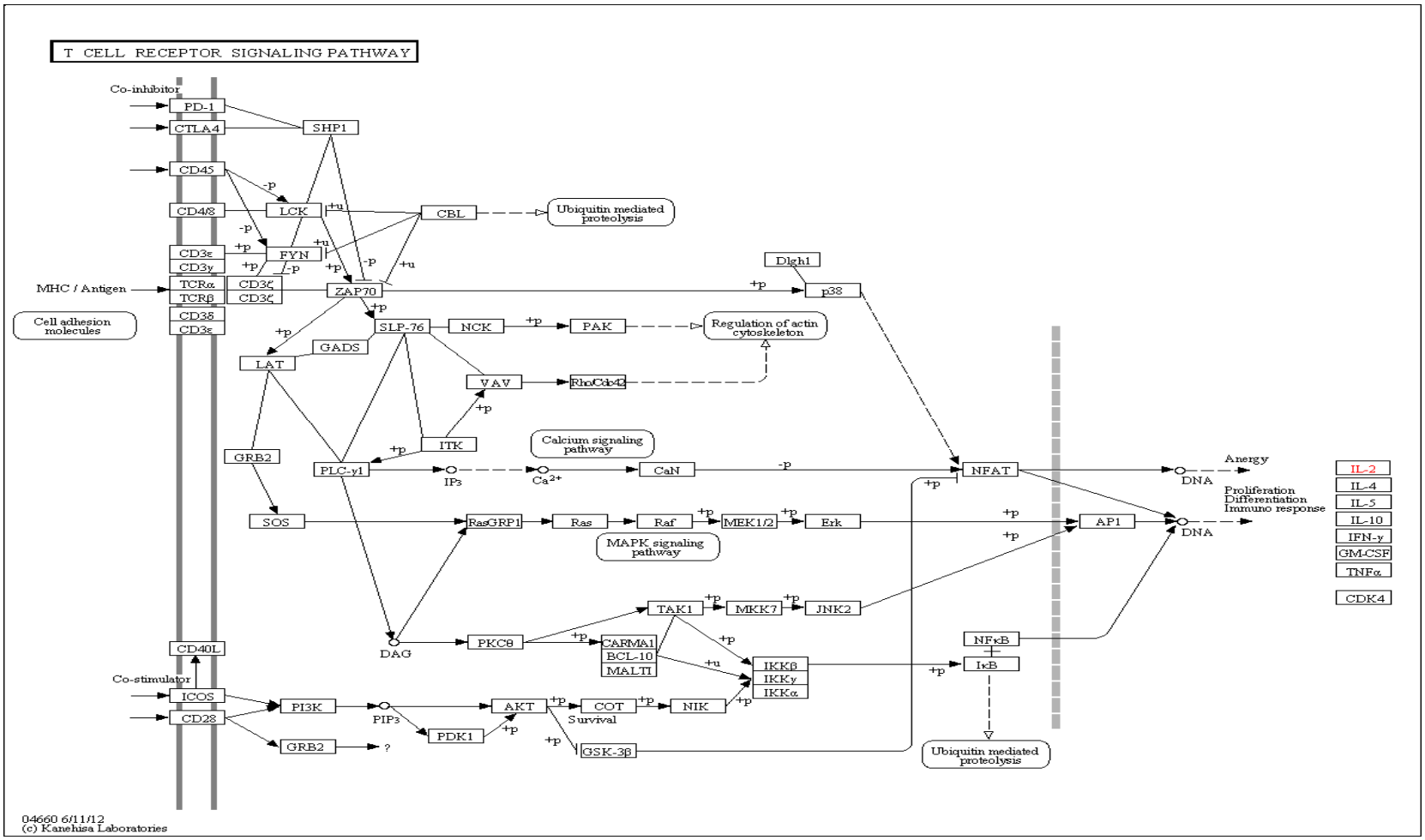


Figure 9 The TCR-APC interaction [77]

The expression of CD44 on the surface of T cells directs these cells to adhere to END cells so that accumulation of lymphocytes at the inflammation site is sustained. Obviously, the expression of CD44 is upregulated in BD [36]. Likewise, the ICAM-1 expression during inflammation is increased and infiltration of neutrophils and T cells through tissues is enhanced. Thus, they argued that increased ICAM-1 yields destruction of keratinocytes in epithelia with the aid of VCAM-1 –causing immune cells to stay close at these sites [36]. In addition to these molecules, other scientists claimed that the chemokine MCP-1 is required for migration of γδ T cells to inflammation sites meaning that a persistent role of MCP-1 is present in BD [37]. The γδ T cells are increased by the presence of active keratinocytes, in response to the MICA recognition [37].

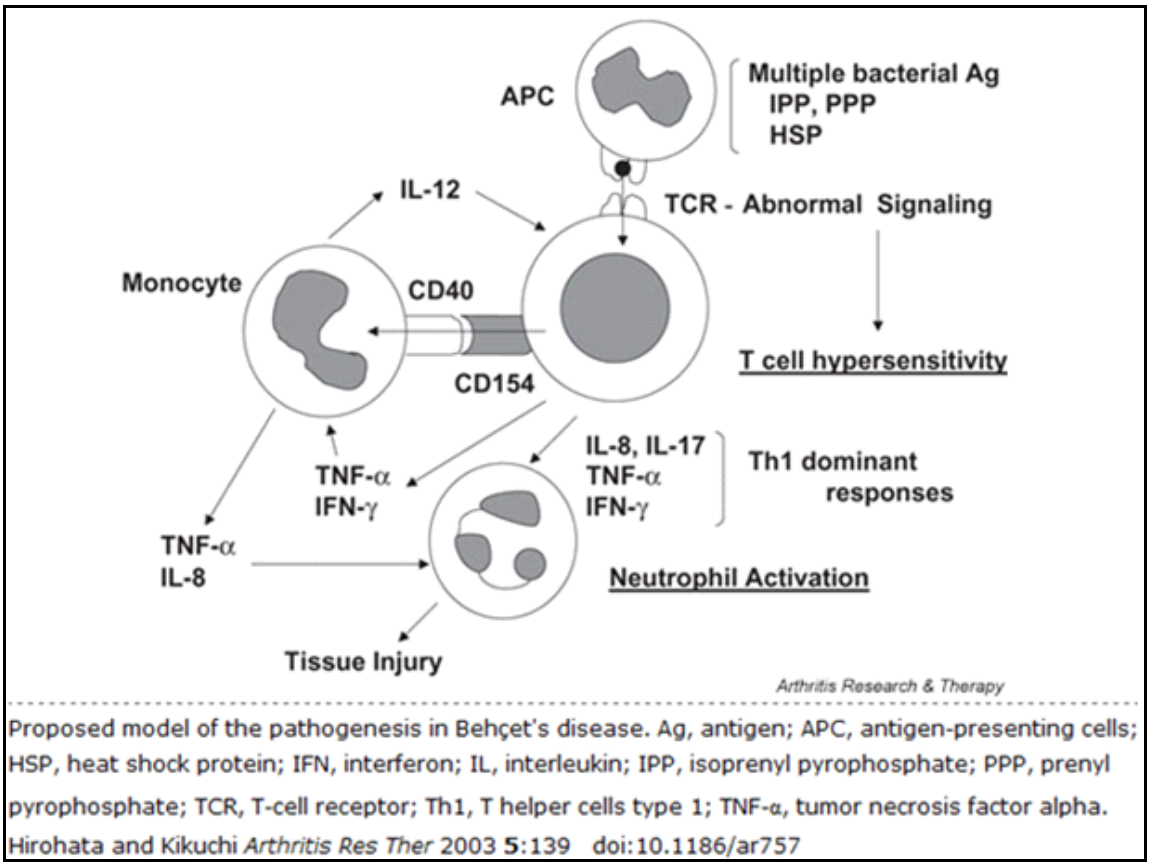
In a Th1 type response, an inefficacy of negative regulation produces less IL-10. The Th1 type cytokines cause a polarization that adaptive immune response, secondary in action, takes place. Moreover supporting the Th1 type polarization, a group of researchers found that CCR5 and CXCR3 receptors are abundant on the cell surface of the lymphocytes [74] whereas Th2 cells express CCR3, CCR4, CCR8 receptors [37].

As conclusion, the paper states that complex interactions among APCs, Th1 lymphocytes, and neutrophils are the basis of the immune aberrations observed in patients with BD [20].

2.5. Pathogenesis Maps

In this part, firstly the possible pathogenesis map of the BD will be described. Some possible maps are already found in the literature like the one shown in Figure 10. As it can be seen from that figure, there are actions of APCs, T-cells, monocytes, and neutrophils. The neutrophil activation is followed by tissue injury and damage. The second map, Figure 11, is adopted from the former, and is proposed by Pay et al. [20]. The latter excludes the

monocytes and is drawn as relations among APCs, T-cells, and neutrophils. Again, the neutrophils activation is shown to lead to tissue damage and inflammation as well as system failures. The cytokines involved in these two figures are tried to be summarized next; their subsequent molecules and actions are given.



Proposed model of the pathogenesis in Behçet's disease. Ag, antigen; APC, antigen-presenting cells; HSP, heat shock protein; IFN, interferon; IL, interleukin; IPP, isoprenyl pyrophosphate; PPP, prenyl pyrophosphate; TCR, T-cell receptor; Th1, T helper cells type 1; TNF-α, tumor necrosis factor alpha. Hirohata and Kikuchi *Arthritis Res Ther* 2003 **5**:139 doi:10.1186/ar757

Figure 10 Possible pathogenesis map of BD, taken from [24]

In Figure 10, the events proposed to occur during BD is summarized where APCs present antigens to the T-cells and a combination of leukocyte actions drives the tissue injury. It is also seen that Th1 dominant response is present where T-cell hypersensitivity leads the way. On the other hand, Figure 11, shows a modified version of the previous figure. The latter discards monocytes, shown in the first, and focuses on the three other cell

types as well as possible antigen sources.

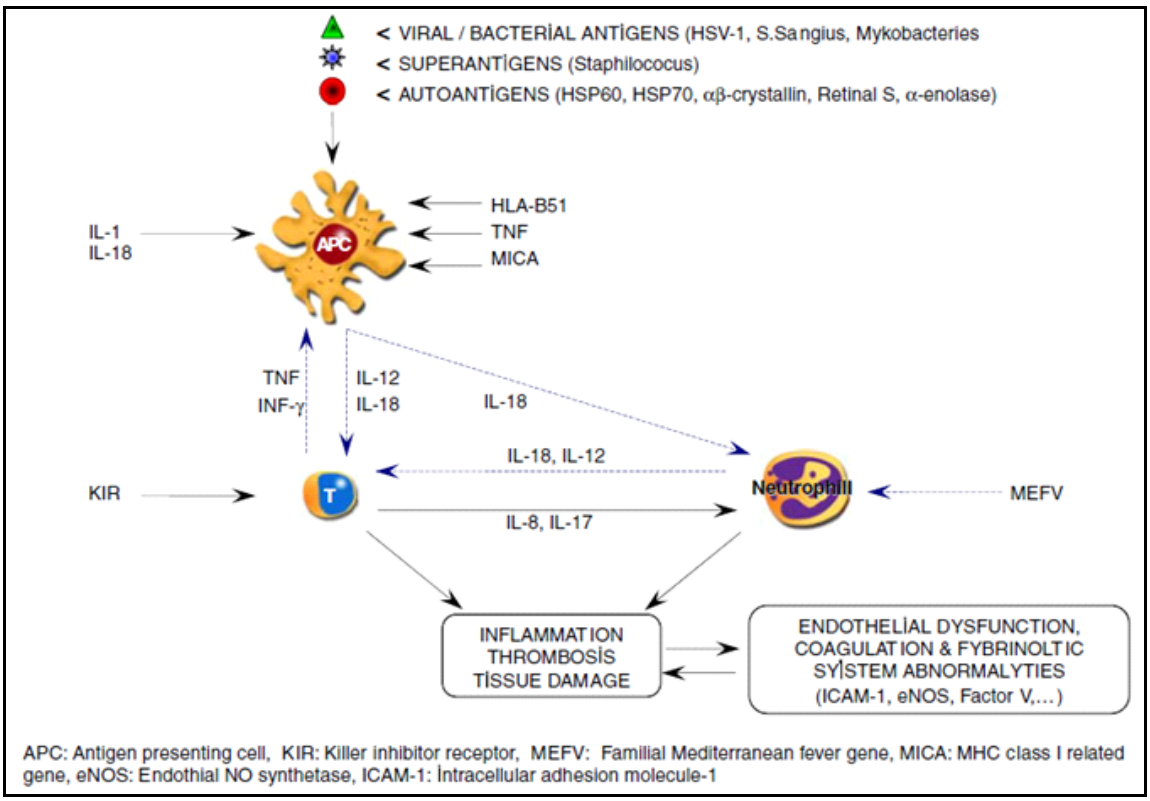


Figure 11 Adapted form of the Figure 10, taken from [20]

Chapter 3

3. METHODS

3.1. Our Pathogenesis Maps

Based on the literature survey and available pathogenesis maps, we constructed a new map depicted in Figure 12. This figure is only a preliminary study, where an overall view of the disease with the intracellular players is shown. In Figure 12, the proposed mechanism of BD starts with a kick by the antigens. The antigens present in the body are uptaken by the APCs and then they are presented on the cell surface for T-cell recognition. Then, when the T-cells and APCs interact, the signal for T-cell maturation and differentiation is initiated. After that, the T-cells release some cytokines: IL-2 for self and neighboring T-cell proliferations, IL-8 for neutrophil activation, and IL-10 for the control of immune reponse types. When IL-10 is present, Th1 type immune response is altered and blocked whereas Th2 type immune response is flared and plasma cells are stimulated to continue on producing antibodies. The stress conditions act through NK-cells and T-cells producing HSPs and MICA for T-cell stimulation. The hyperactivation of neutrophils with the action of T-cells, tissue damage and inflammation occur causing the disease to unfold. In Figure 12, the elements have their names written within where the green arrows mean either one of production, release, or positive action and the red arrows mean the inhibition of the targeted green arrow action. The molecules written above and below the arrows are the corresponding elements produced.

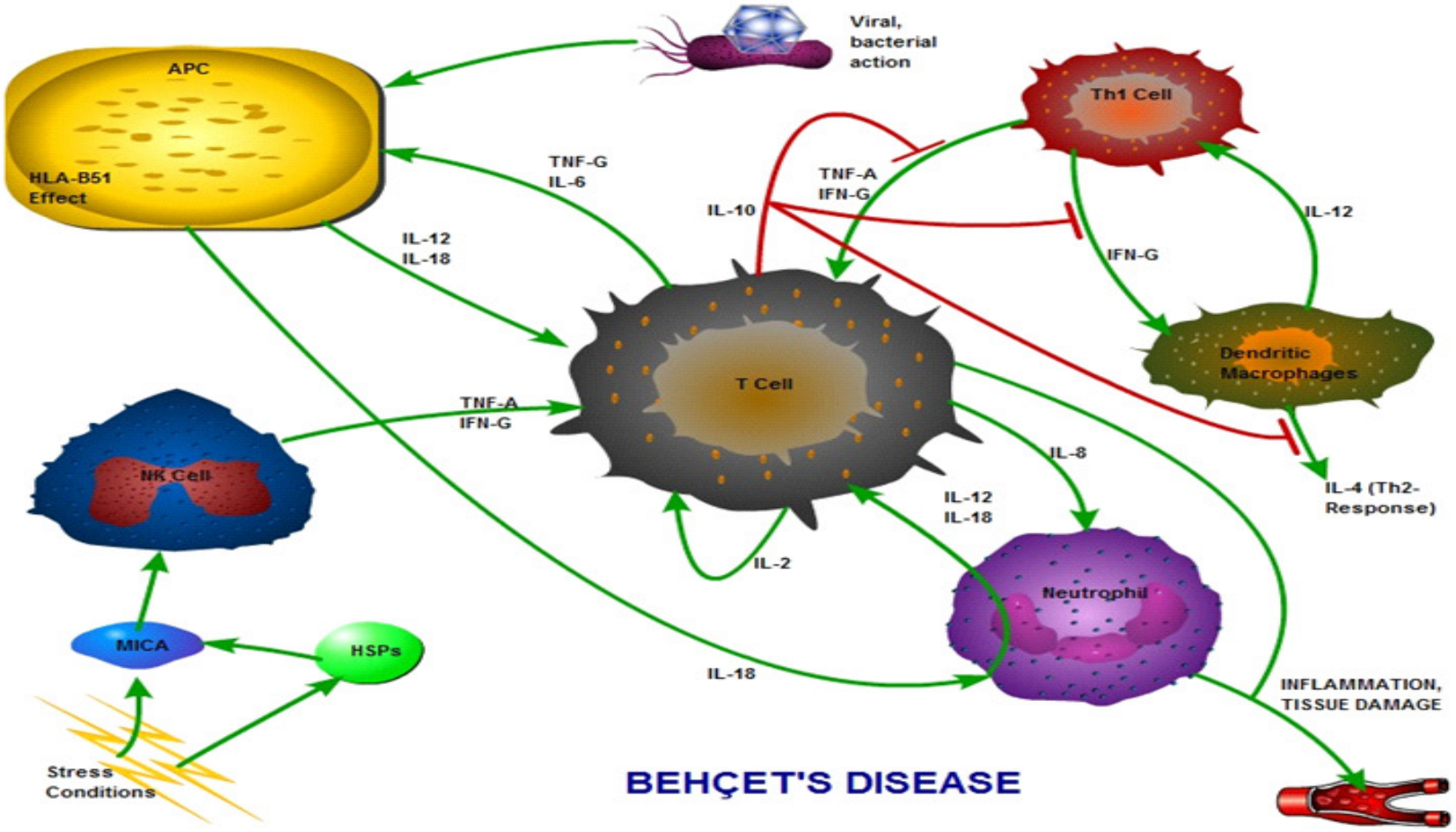


Figure 12 The first model of the disease

After the first model, Figure 12, a new comprehensive model is constructed. This new model is given in Figure 14 and it contains more, detailed information. The steps of incidents presented in Figure 14 are listed in Box 1.

Box 1 The events employed in pathogenesis map 2, Figure 14	
1	Due to the action of HLA-B*51 or of any antigen, APCs represent peptide fragments on their surface via their specific surface MHC proteins.
2	These MHC-peptide complexes are recognized by naïve T cells.
3	TCR (T cell Receptor)-APC interactions occur.
4	IL-2 synthesis is started together with some other cytokines.
5	APCs also release IL-12 and IL-18 to T cells to produce IFN-G.
6	APCs additionally stimulate neutrophils to produce IL-12 and IL-18, which stimulate T cells for TNF-A and IFN-G production.
7	IL-8 and IL-17 from T cells cause neutrophils to migrate to inflammation sites and cause hyperactivation.
8	T cells also release TNF-A and IFN-G to stimulate APCs as well as to prime neutrophils and hyperactivate.
9	Activated neutrophils release more superoxides and ROS causing oxidative stress.
10	Finally on neutrophils, HSP-60 epitope 336-51 peptide results in production of IL-12, TNF-A, and IFN-G.
11	The production of IL-2 in T cells results in synthesis of more IL-2, IL-4, and IFN-G, leading further growth and Th1 type response.
12	HSP-60/65 (epitope 336-51) activates T cells to produce more TNF-A and IFN-G.
13	IL-12 and IL-18 from T cells cause NK cells to enhance their killing activity and to produce IFN-G and TNF-A, binding to T cells.
14	TNF-A and IFN-G from NK cells cause T cells to produce IL-12 and IL-18, which stimulate NK cells.
15	Streptococcus and E.Coli stimulation of T cells results in IFN-G and IL-6 synthesis.
16	IL-8 and MCP1 cause END Cells to bind to monocytes more firmly.

17	Moreover, HSP70 binding at TLRs of the END Cells cause more cytokine synthesis from those cells.
18	Cytokine regulated Leptin causes NO production from END Cells.
19	Moreover, E-selectin levels at END surface increase causing more infiltration of immune cells.
20	A-enolase and MICA expressions by END Cells are also increased causing more END injury.
21	CXCR3 expression of END Cells increased, resulting in more firm binding of T cells.
22	At last more ICAM-1 is also synthesized in response to some antigens.
23	Stress conditions in the body cause more HSP and MICA expression and MICA- which is mostly synthesized in END Cells, is recognized by NK cells.
24	HSP expression is also increased by mucous cells in response to bacterial stimuli.
25	MICA induces stimulation and action of NK cells, where they produce IL-4 at early stages of immune response.
26	In BD Th1 type response is dominated where T cells tend towards Th1 cell type, which is supported by presence of IFN-G but inhibited by both IL-4 and IL-10. On the other hand, IFN-G represses a response towards Th2 type whereas IL-4, which is also produced by Th2 type T cells, enhances that tendency. IL-10 also inhibits Th2 type response.

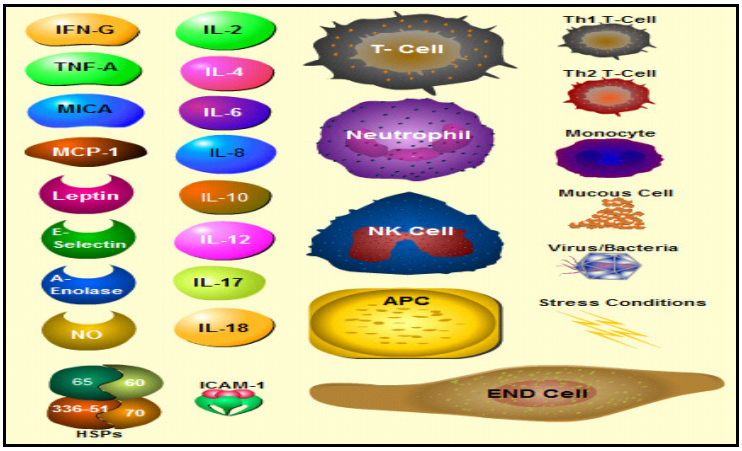


Figure 13 The legend for second model (Figure 14)

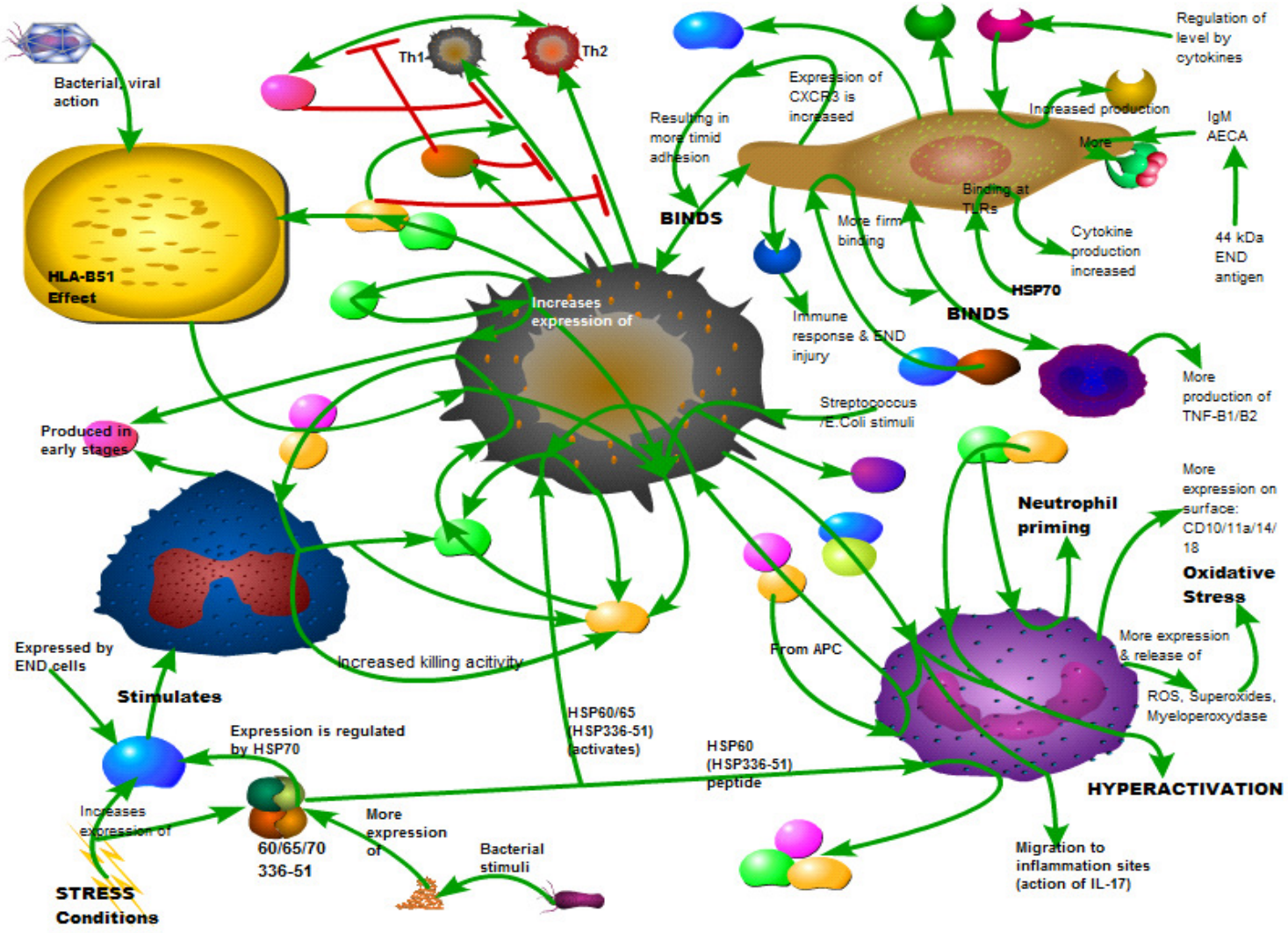


Figure 14 The second and more detailed model of the disease

The legend for the second model is given in Figure 13. The arrow usage is the same in Figures 12 and 14. Although this model is very comprehensive and detailed, it lacked some critical information that is not present in published literature so far. Thus the second figure was modified and simplified yielding the drawing in Figure 15.

3.2. Final Pathogenesis Map

There are some pathogenesis maps on how BD may progress [20, 24], however, they are incomplete and insufficient to provide detailed insight into BD. In Figure 15, our understanding and model for the disease development is shown. The three primary variables of our model are the quantities of FLD (first line defense, i.e., innate immune, cells), SLD (second line defense, i.e., adaptive immune, cells), and HLA-B51 as shown in the figure. Based on the interactions depicted in Figure 15, a flow of progression and mechanism is deduced for the stages of BD advancement. Figure 16 summarizes the flow.

BD is known to be affected by the presence of HLA-B51 so that any trigger may start an inflammation, which leads to recurrent attacks of the immune cells in the body. These attacks are primarily by the innate immune cells, neutrophils and natural killer cells (NK cells). Furthermore, the adaptive immune response also takes part in these flare phases. Due to the malfunctions in the control mechanism of the immune responses, high numbers of killer cells are produced, which in turn cause tissue damage. In Figure 16, the proposed mechanism of the disease propagation is given. The rest of the study is based on these steps and the relations in between. These steps are both derived from the comments of Prof. Ahmet Gül and the literature review. Based on the preliminary data obtained and presented above, the first pathway model is drawn. Figure 15 shows the basic steps and molecules produced and secreted among different cell types.

The process of the third model is given in Box 2.

Box 2 The events employed in pathogenesis map 3, Figure 15	
1	The triggers interact with the FLD, which is the collection of antigen presenting cells, endothelial cells, and keratinocytes. The triggers may comprise but not limited to bacterial-viral components, HSPs, and MICA.
2	This interaction, which is also effected by HLA-B*51, causes pro-inflammatory cytokine release as well as neutrophil/monocyte collection at the inflammation site.
3	Together with the effects of triggers and HLA-B*51, the SLD (which is composed of T lymphocytes, NK cells, neutrophils, and monocytes) interacts with FLD. The results of this interaction are: (1) more ProC release, (2) more activation of themselves, (3) release of IL-12 and IFN-G, (4) release of IL-10 (+ some other), and (5) production of superoxides/autoantigens-leading to further triggering.
4	The neutrophils then produce molecules like S100A12, which causes more expression of TNF-A. Being one of the ProC, TNF-A then causes more S100A12 production.
5	Adhesion molecules ICAM-1 and VCAM-1 are also produced by FLD in response to S100A12.
6	The presence of more adhesion molecules results in destruction of more cells thus more inflammation and tissue damage.
7	The IL-12 and IFH-G produced are of type Th1, and they cause an immune response of type Th1, which is downregulated by IL-10 (together with some other).
8	Together with hyperactivity of neutrophils, the Th1 cells induce an elevated immune response in BD.

Using the data present in the third model, the equation system was formulated. The system will be represented by a system of Ordinary Differential Equations (ODEs), where, at first, only two equations were written. However, the two equations were found to be deficient in giving the desired behavior of the disease, a third equation was formed. The section below summarizes this process of ODE system construction.

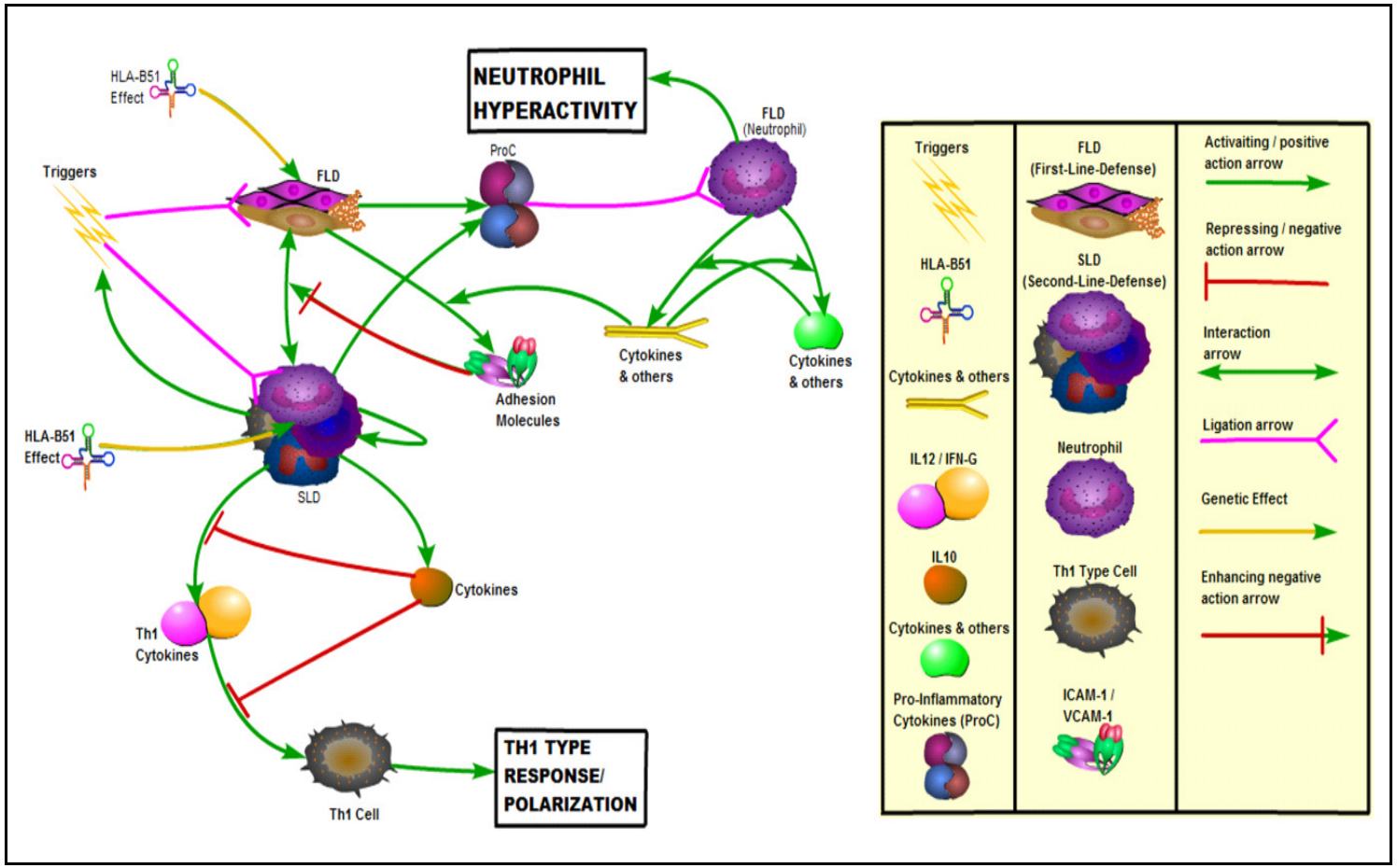


Figure 15 The model of the disease pathogenesis.
FLD: First line defense (innate immune) cells. **SLD:** Second line defense (adaptive immune) cells.

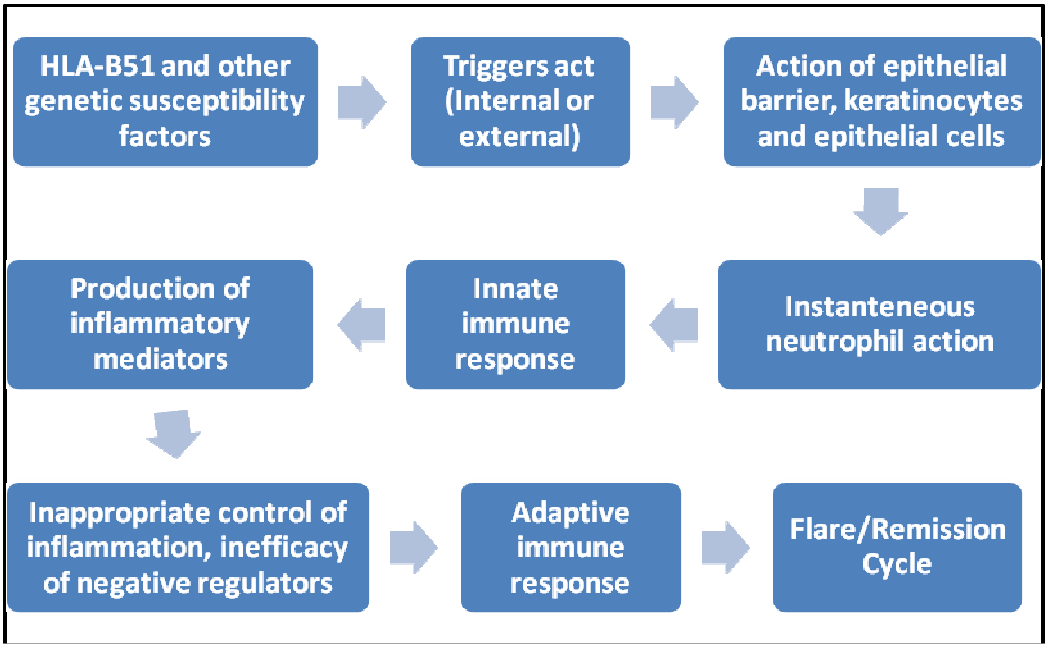


Figure 16 The proposed disease progression steps

3.3. The Mathematical Models

3.3.1. The Dynamical Model of the Diseased

The state variables of our model are the quantities (in arbitrary units) of HLA-B51 denoted by H, the adaptive immune (basically T) cells denoted by S, and the innate immune (neutrophils, NK, macrophages) cells denoted by F. The three-variable ODE system is deduced from the reaction system given in Table 1. The parameters have no defined units because we used lumped actions of many cells and other organic materials in construction of our models. Thus, any parameter will have corresponding units associated with concentrations of the state variables. A similar approach is also followed in [30].

Table 3 The reaction system of the BD pathogenesis

Rxn 1.	$Trig + H \xrightarrow{k1} 2 \cdot H + F$	The activation of HLA molecules by triggers and subsequent innate immune cell activation
Rxn 2.	$F \xrightarrow{k2} \alpha \cdot S$	Activation of adaptive immune cells by innate immune cells
Rxn 3.	$H + S \xrightarrow{k3} P$	Killing of infected cells by adaptive immune cells
Rxn 4.	$Trig + S \xrightarrow{k4} H + P$	Further activation of HLA molecules by the actions of adaptive immune cells
Rxn 5.	$H + H \xrightarrow{k5} P$	Collective killing of infected cells by any means

$\boldsymbol{H}$: *HLA-B51 proteins.* $\boldsymbol{S}$: *Adaptive immune cells.* $\boldsymbol{F}$: *Innate immune cells.* **T**: *Triggers.* $\boldsymbol{P}$: *Damaged cells.*

According to our model, represented by the reaction system in Table 1, when the triggers (the pathogens or self antigens) are present in the body, the HLA-B51 proteins manifest two different actions leading to inflammation, as captured by Reaction 1. The first of these actions is the non-specific representation of protein fragments to immune cells, and the second is the misfolding and accumulation of these distorted HLA proteins in the endoplasmic reticulum, causing ER stress [4]. Moreover, these two roles of HLA and antigen complexes cause an immediate and elevated response of innate immune cells, $\boldsymbol{F}$. Then, as captured by Reaction 2, when the innate immune cells are activated and accumulated at the inflammation sites, they produce cytokines and the secondary (adaptive) immune cells are subsequently activated. The parameter $\boldsymbol{\alpha}$ in this reaction can be used to tune the interactions of the different immune cell types in the system. In addition, as represented by Reaction 3 in Table 1, the already present HLA proteins on the cell surfaces, complexed with an antigen, interact with $\boldsymbol{S}$ cells resulting in the death of the infected cells. In the vicinity of cytokines produced by $\boldsymbol{S}$ cells, some of the $\boldsymbol{F}$ cells, macrophages, become more active when $\boldsymbol{S}$ cells are activated in the presence of the triggers, as represented by Reaction 4. Moreover, in this cascade, the infected cells and the pathogens are killed or

eliminated. Finally, as HLA protein plus antigen complexes accumulate on the cell surfaces, they are attacked by different levels of the immune system and are eliminated, as shown by Reaction 5. Based on the above and the reactions in Table 1, a three variable ODE system can be constructed using the mass-action law. The three equations are listed below in (1):

$$\left(\frac{dH}{dt}\right) = k_1 \cdot [T] \cdot [H] - k_3 \cdot [H] \cdot [S] + k_4 \cdot [T] \cdot [S] - 2 \cdot k_5 \cdot [H]^2$$

$$\left(\frac{dS}{dt}\right) = k_2 \cdot \alpha \cdot [F] - k_3 \cdot [H] \cdot [S] - k_4 \cdot [T] \cdot [S] \quad (1)$$

$$\left(\frac{dF}{dt}\right) = k_1 \cdot [T] \cdot [H] - k_2 \cdot [F]$$

Next, the ODE system above is converted to a dimensionless form following the same steps for the Oregonator system described in [79], and using the definitions given in (1.1).

$$h = \frac{H}{H_0} \qquad s = \frac{S}{S_0} \qquad f = \frac{F}{F_0} \qquad \tau = \frac{t}{t_0}$$

$$H_0 = \frac{k_1 \cdot T}{2 \cdot k_5} \qquad S_0 = \frac{k_1 \cdot T}{k_3} \qquad F_0 = \frac{(k_1 \cdot T)^2}{k_2 \cdot k_5} \qquad t_0 = \frac{1}{k_2} \quad (1.1)$$

$$\gamma = 2 \cdot \alpha \qquad \theta = \frac{1}{2} \qquad q = \frac{2 \cdot k_4 \cdot k_5}{k_1 \cdot k_3} \qquad \varepsilon_1 = \frac{k_2}{k_1 \cdot T} \qquad \varepsilon_2 = \frac{2 \cdot k_2 \cdot k_5}{k_1 \cdot k_3 \cdot T}$$

In (1.1), the parameters with subscript zero refer to reference conditions. h, s, and f are the state variables in normalized form. The rest are some constants defined for conciseness. The new dimensionless model of the diseased system is given by the equations in (2) below:

$$\varepsilon_1 \cdot \left(\frac{dh}{d\tau}\right) = (q \cdot s - h \cdot s + h - h^2)$$

$$\varepsilon_2 \cdot \left(\frac{ds}{d\tau}\right) = (\gamma \cdot f - h \cdot s - q \cdot s) \tag{2}$$

$$\left(\frac{df}{d\tau}\right) = (\theta \cdot h - f)$$

The above dimensionless system has three fixed points (obtained by setting the derivatives with respect to time to zero), one of which is the all zero point and two others. The expressions for these fixed points are given in Appendix A. One of the other fixed points is negative and it is physiologically meaningless. The zero fixed point is always stable. The other nonzero, physiologically meaningful fixed point is a function of γ, θ, and q only, independent of the amount of trigger, T. The steady state solution of Equation (2) is obtained by setting the left-hand-side to zero and solving for s, in terms of h and s with $\varepsilon_1 \gg \varepsilon_2$ (see [79]). This is, then, substituted into the first equation in (2). The final reduced form of the ODE system is given below in (3):

$$s = \frac{\gamma \cdot f}{q+h}$$

$$\varepsilon_1 \cdot \left(\frac{dh}{d\tau}\right) = \left(q \cdot \left(\frac{\gamma \cdot f}{q+h}\right) - h \cdot \left(\frac{\gamma \cdot f}{q+h}\right) + h - h^2\right) = \gamma \cdot f \cdot \left(\frac{q-h}{q+h}\right) + h - h^2 \tag{3}$$

$$\left(\frac{df}{d\tau}\right) = (\theta \cdot h - f)$$

There are three fixed points for the model in (2): a positive, a negative, and an all-zero one. The Jacobian of the two dimensional nonlinear function that defines the RHS of the two differential equations in (3) is computed and given in Appendix B. The trace of this Jacobian (equal to the sum of its two eigenvalues) is also evaluated and given in (4). These two equations for the Jacobian and its trace are generic, and appropriate fixed point data is to be substituted whenever needed.

$$trace(J_{ss}) = \frac{\left(1 - 2 \cdot h_{ss} - \frac{2\gamma q f_{ss}}{(q+h_{ss})^2}\right)}{\varepsilon_1} - 1 \quad (4)$$

In (4), the subscript "ss" indicates that the corresponding variable is calculated at a steady state fixed point given in Appendix A. Here, when the value of the trace in (4) is less than zero (eigenvalues with negative real parts), the corresponding fixed point for the system is stable, and when the trace is greater than zero (eigenvalues with positive real parts), the fixed point is unstable [79]. When the trace is equal to zero (eigenvalues with zero real parts), it corresponds to a Hopf bifurcation [79], corresponding to a stability-instability boundary. Thus, the trace in (4) is set to zero and ε_1 is solved in terms of the other parameters after which the definition of ε_1, γ, q, h_{ss} and f_{ss} are substituted for the positive fixed point of the three-variable model. Then, the T in ε_1 is solved for in terms of all the other parameters, which enables us to plot T as a function of k_1, as shown in Figure 17.

In Figure 17, in the parameter region on the left of the red line, the trace is less than zero, the fixed point is stable and as a result there is no limit cycle and hence no steady oscillations. However, on the right side of the red line and above the blue curve, the fixed point is unstable and it is encircled by a limit cycle resulting in steady oscillatory behavior. On the other hand, the region below the blue curve but on the right of the red line also corresponds to a stable fixed point. One can conclude that below a certain value of the rate constant of Reaction 1 in Table 1 (k_1), the system has a stable fixed point meaning that the triggers are eliminated and the immune cell numbers settle down to steady values. The rate constant k_1 reflects the various actions of the HLA proteins. If k_1 is above a critical value, recurrent attacks become possible depending on the amount of triggers. For every value of k_1, there is a critical trigger amount above which recurrent attacks occur. This critical trigger amount depends on the value of k_1, and it decreases rapidly for increasing values of k_1.

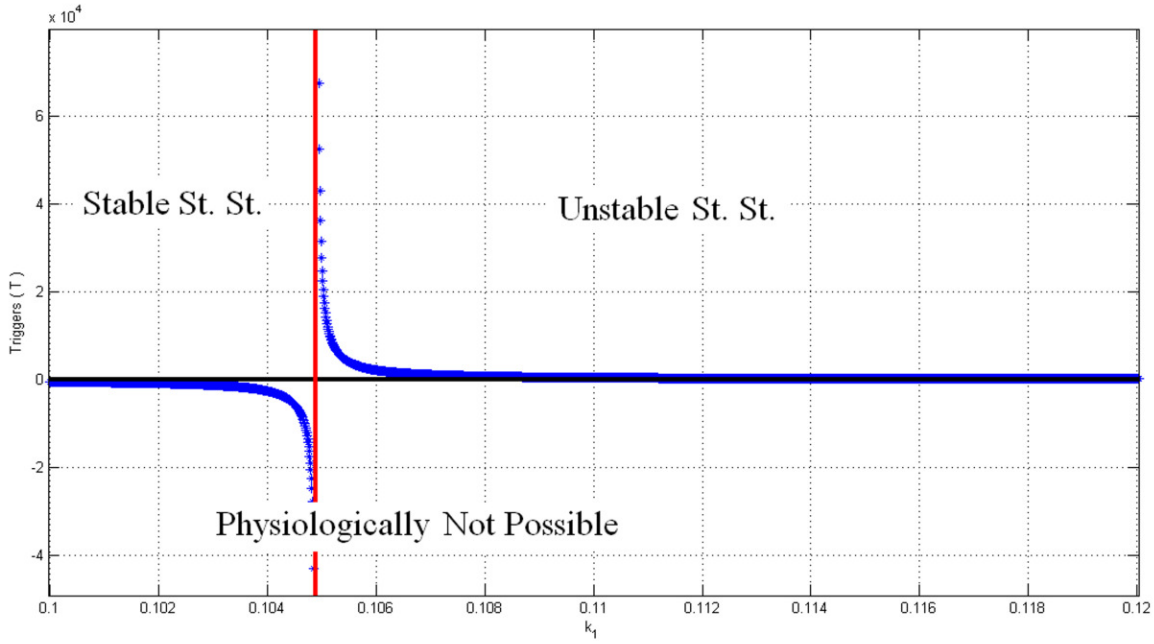


Figure 17 The trigger amount versus rate constant for Reaction 1 in Table 1 (T vs. k_1). Considering only the positive valued quadrants, the unstable region is above the blue curve in the right quadrant, whereas the stable region is on the left quadrant and also below the blue curve of the right quadrant. The negative valued quadrants are not physiologically meaningful. The figure is obtained using the following parameter values constant:
$k_2 = 1.0, k_3 = 10^6, k_4 = 2.0, k_5 = 2000, \gamma = 2.0, \theta = 0.5$.

Another bifurcation analysis for the pair T vs. k_3 is also carried out in a similar manner, which is shown in Figure 18. The behavior in Figure 18 is similar to the one in Figure 17. This plot captures the effect of the rate of interactions of infected cells (cells with "H" with the secondary immune cells, and the trigger amount. This result may correspond to the fact that patients of BD express an elevated Th1 type response of T-cells. Thus, one may argue that the regulation of T-cell activity in the disease, especially in the phase of interaction of these T-cells with the infected cells may constitute one major decision point whether the immune system responses is recurrent or not. For small values of k_3, the system is stable and does not exhibit recurrent behavior.

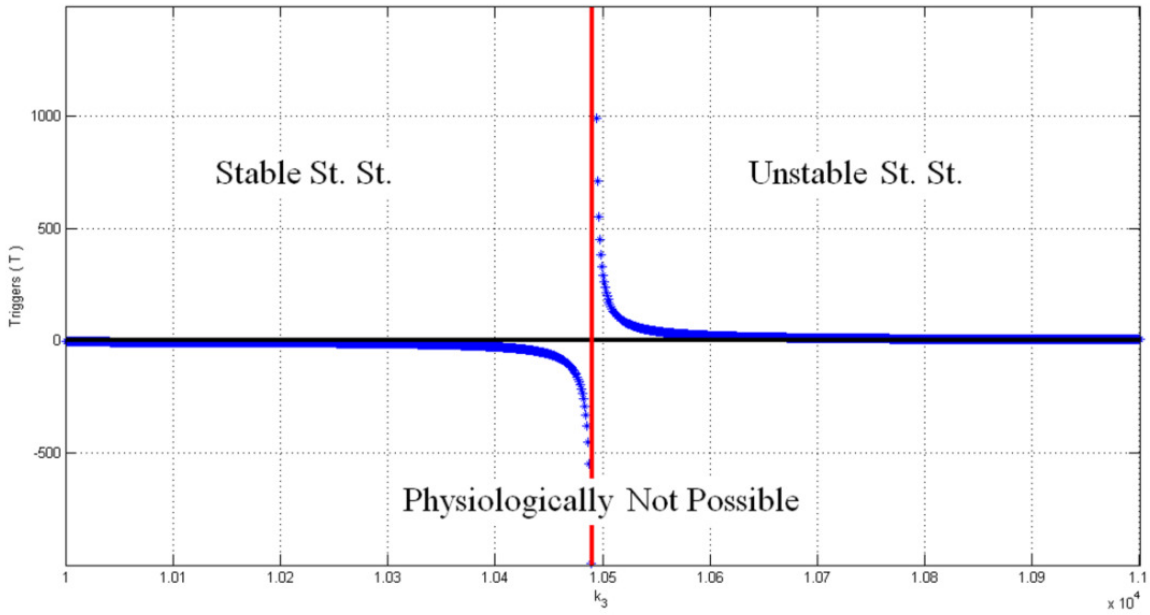


Figure 18 The bifurcation diagram for parameters (T vs. k_3).
Considering only the positive valued quadrants, the unstable region is above the blue curve in the right quadrant, whereas the stable region is on the left quadrant and also below the blue curve of the right quadrant. The negative valued quadrants are not physiologically meaningful. The figure is obtained using the following parameter values constant:
$k_1 = 10, k_2 = 1.0, k_4 = 2.0, k_5 = 2000, \gamma = 2.0, \theta = 0.5$.

To sum up, if a person is the carrier of HLA-B51 and has high values of k_1, and/or k_3, s/he will probably demonstrate the symptoms of BD. For lower values of these rate constants or under fairly low antigenic conditions, one may not suffer from recurrent attacks of inflammation although being a carrier of HLA-B51.

The steps followed in the methods are summarized in Box 3.

Box 3 The steps followed for analysis of our diseased model	
1	The three-variable model is constructed
2	Fixed points of this original form is evaluated (There are three: positive, negative,

	and all-zero)
3	The three-variable model is normalized using a procedure similar to that of Oregonator models
4	Based on this new normalized form, the system is reduced to a two-variable model
5	The Jacobian of this reduced form is calculated
6	The Trace of this Jacobian (of reduced form) is calculated
7	The positive valued root of the three-variable model and all other definitions of the parameters are substituted in this Trace equality
8	The trigger amount, T, is solved in terms of all other parameters using the equation obtained in the previous step
9	Figures 17 & 18 are plotted using the equation for T
10	Finally, using the value ranges of parameters from the distinct regions in these two plots (Figures 17 & 18), the simulations are carried out using the three-variable model.

3.3.2. The Dynamical Model of the Healthy

For people having serotypes other than HLA-B51, Reaction 1 in Table 1 can be recast as:

$$Trig + H \xrightarrow{k1} H + F \tag{5}$$

Using the system in Table 1 with the above reaction replacing the first, we have gone through all the steps up to the calculation of the trace of the Jacobian for the fixed

points. The steps followed for this healthy system is exactly the same as that of the diseased model, presented in Box 3. This analysis reveals that the trace is always negative for physiologically sound values of the parameters. Thus, the system always has stable fixed points, with no possibility of steady oscillations or recurrent behavior, for all the values of triggers or k_1 or any other parameters (for their positive values).

$$trace\_new(J_{ss}) = \frac{\left(-2 \cdot h_{ss} - \frac{2 \cdot r \cdot q \cdot f_{ss}}{(q + h_{ss})^2}\right)}{\varepsilon_1} - 1 \quad (6)$$

The equality (6) shows that, if the constants and the values of h_{ss} and f_{ss} are positive, then the trace is negative, meaning that the system always has stable fixed points. Thus, we can represent the healthy population, or people with HLA proteins other than HLA-B51, with this modified system of equations. This new system is in accordance with the work by Reynolds et al. [30], which will be examined in the next section.

Chapter 4

4. Results

4.1. The Healthy System

Our healthy system model differs from the diseased system only by the increased and enhanced activity of HLA-B51 in the population at risk in the latter system. The dynamical system model of the healthy should exhibit the normal response of the immune system against infection. We investigated our healthy system model behavior in response to a step and a pulse input of triggers, as shown in Figure 19. In Figure 19a, a pulse input of the pathogen is initiated at t = 26. An immense immune cell respond follows the increase of active HLA complexes formed due to the trigger pulse. When the pathogen level returns to normal, the immune cell counts settle down to its previous fixed point also. In Figure 19b, however, a step input of the pathogen (at t = 26), i.e., with a continued infection, is applied and the immune system responds immediately and the numbers of both innate and adaptive immune cells stay elevated in an alert state as long as the pathogen introduction continues.

According to Reynolds et al. [30] there are three modes of the anti-inflammation process, which are the healthy, the aseptic death, and the septic death. In the healthy case, the intruder pathogens are cleared by immune cells completely, as such all the variables of their model settle to zero (except for the concentration of mediators of anti-inflammation); i.e., the number of pathogens as well as that of the immune cells. In the aseptic death case, the pathogen is cleared but the number of immune cells stay elevated and they cause tissue damage. This second fixed point has a non-zero value only for immune cells. Finally, the

septic death mode corresponds to pathogen clearance failure and thus continued immune response, with a fixed point of non-zero values for both the immune cells and the pathogens.

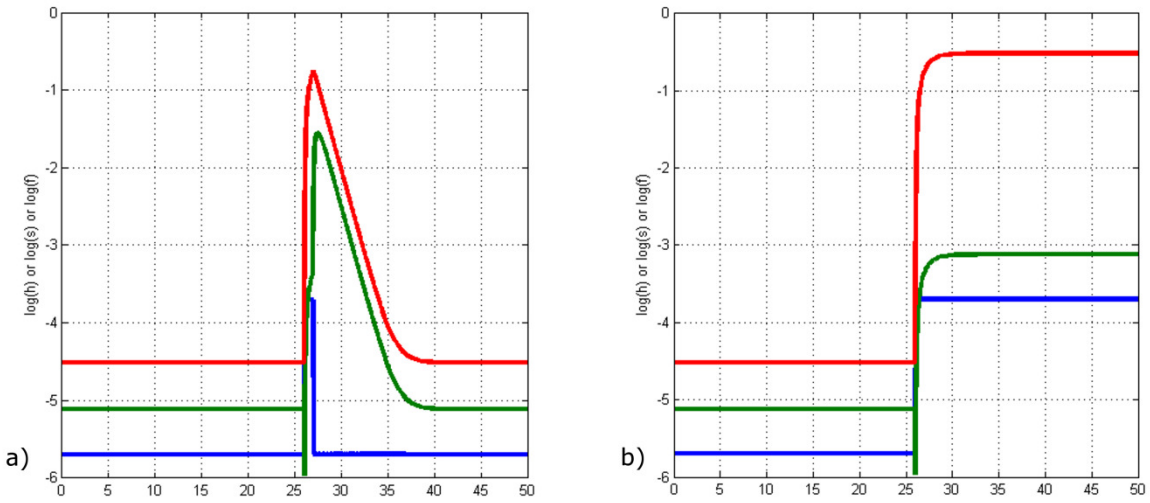


Figure 19 The behavior of the healthy model when a pulse and step input of triggers is applied.
The constant parameters for all cases are: k_1= 15.23, k_2 = 1.0, k_3 = 10^6, k_4 = 2, k_5 = 2000. The T is changed from 1 to 100 at the input times, in both cases.

To further test our healthy system model, we studied the anti-inflammation modes described by Reynolds et al. [30] and the results are depicted in Figure 20. In the healthy case, when there was a certain amount of pathogen at the start and no immune cells, the values of our three variables approach to a fixed point, which is very close to zero and taken as the baseline. When the system is at this fixed point, a pulse input of pathogens is given at t = 26, and the system settles back to the starting fixed point, as shown in column (a) in Figure 20. The three plots in the column (b) in Figure 20 show the trend of our state variables in the aseptic case. Starting with a higher pathogen amount compared to health case in column (a), our variables settle a fixed point with non-zero immune cell values, 100 times higher innate immune cells and 10 times more adaptive immune cells than those of

the health mode. When we apply a pulse input of pathogen at t = 26 to this system, it comes back to its fixed point after a normal immune response. In this case, tissue damage occurs due to elevated immune cell counts. The case of septic death is illustrated in the third column, part (c), of Figure 20. When we started a simulation with a lower value for k_3 (lower k_3 results in higher h values), our system approached to a fixed point of all-non zero condition: in addition to the immune cells, the infected cells also stay elevated in the system. If a pulse input of triggers is given at t = 26 at this septic fixed point, the system returns to that point after the effect of the pulse is eliminated. The values of all three variables of our system are almost 1000 times higher in this septic mode fixed point compared to the fixed point in health mode.

4.2. The Diseased System

In our diseased system model, the system has three modes: Carrier (of HLA-B51) with no BD symptoms, a potent patient, or a patient with recurrent attacks, resulting from the bifurcation analyses shown in Figures 17 and 18. The two plots of Figure 21a are the behaviors of the carrier with no BD symptoms, which are similar to the health mode in our healthy system model shown in Figure 19. The system is at the carrier but healthy mode of the diseased in Figure 21a. The system has a positive steady state ($h = 2.3 \times 10^{-6}$, $s = 5.4 \times 10^{-9}$, $f = 2.3 \times 10^{-8}$) and when a pulse input of triggers is applied, a normal immune response occurs, after which the system variables settle down to the previous fixed point. At the upper right corner this plot, $log\ (h)$ curve is magnified for better inspection. In the second plot of Figure 21a, however, the system is under a step input of triggers starting at t = 51. The system acquires a new steady state and stays there as long as the new trigger amount persists.

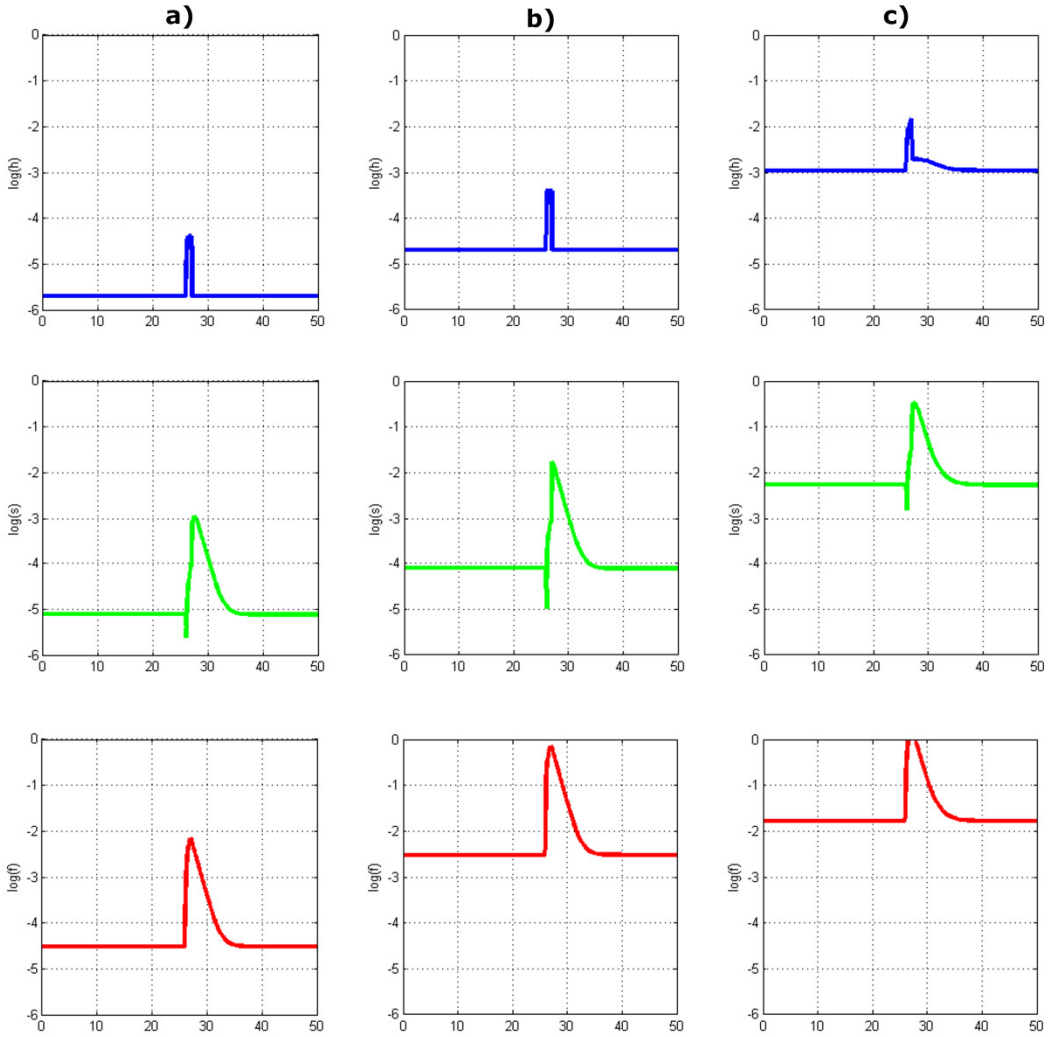


Figure 20 The behavior of the healthy model: Healthy state, aseptic death, septic death cases.
Initial conditions for all cases are: $h_0 = 0.125$, $s_0 = 0.0$, $f_0 = 0.0$. The constant parameters for all cases are: k_1= 15.23, k_2 = 1.0, $k_4 = 2$, $k_5 = 2000$. T is changed in (a) from 1 to 20, in (b) from 10 to 200, and in (c) from 1 to 20 at the times of pulses. k_3 is (a & b) 10^6, and (c) 10^3.

In the potent patient case, the rate constant k_1 is larger than its critical value but is still under the blue curve. In Figure 21b, the potent patient enters the diseased zone when the trigger level is increased from 1 to 100 at t = 51. A temporary transition to disease symptoms is sustained until the trigger amount is decreased to 1 at t = 72. On the other hand, the recurrent inflammation attacks continue to occur unless the trigger exposure amount is restored to a lower value, as shown in the second plot of Figure 21b (a step input is introduced at t = 51).

In Figure 21c, a system in the diseased mode is illustrated. At a constant k_1 value, the trigger amount (T) needed to cause continuous immune attacks should at least be on the blue curve illustrated in Figure 17. As shown by the plots in Figures 21c and 22, although the trigger or pathogen exposure is kept constant, the numbers of the all immune cells and the cells presenting HLA-B51 oscillate. At t = 75, a pulse input of triggers is applied on this oscillating system and the immune system flares a greater but transient immune response. The system returns to its previous course of action after the elevated pathogen level is restored to normal. In the diseased mode, a step input of triggers at t = 75 shifts the values of system variables to a higher level, where they continue to oscillate at a higher frequency; a decrease of two time units in the periods of oscillations occurred after the step input.

When the diseased and the healthy system models are compared, in the healthy system model, there are no immune attacks if no pulse or step input of pathogens are present, Figure 19. On the contrary, the diseased system has auto-inflammation at constant trigger exposure even if there is no further pathogen attack, Figure 22. The infected cell counts continue to rise until a point, where the immune cell attacks start and then the remission takes place, Figure 22.

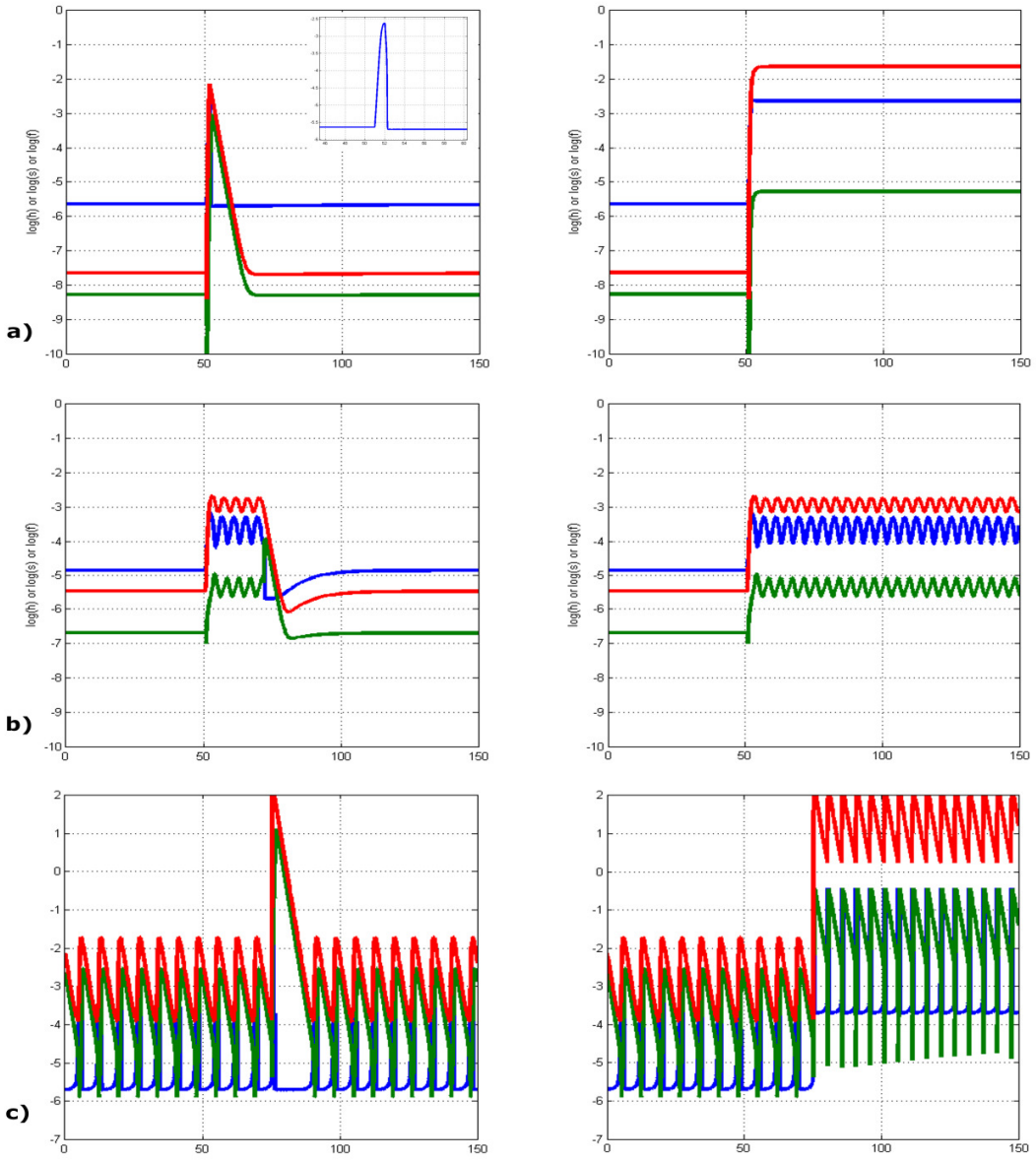


Figure 21 Simulations of the diseased system model.
Parts (a), (b), and (c) represent the healthy carrier, the potent patient, and the diseased individual respectively. The constant parameters for all cases are: $k_2 = 1.0$, $k_3 = 10^6$, $k_4 = 2.0$, $k_5 = 2000$, $\gamma = 2.0$, $\theta = 0.5$. T is changed in (a) from 1 to 1000 and in (b & c) from 1 to 100 at the times of inputs. k_1 is 0.01, 0.23, and 15.23 in (a), (b), and (c), respectively.

A closer look at the diseased mode, which is the major concern of paper, is needed and this mode without any transient inputs is illustrated in Figure 22. In such systems, the number of cells with HLA proteins or the infected cells increase with time and reach a peak at some point. Then the first line defense cells increase in number and they produce cytokines to stimulate the secondary immune cells. When these adaptive immune cells come into action, they cause the infected cells to decrease dramatically, after which the infected cells start to accumulate again. The accumulation of the pathogens and hence infected cells causes another inflammation attack. Thus, the flare/remission cycles of inflammatory attacks in Behçet's Disease are obviously captured by our diseased mode.

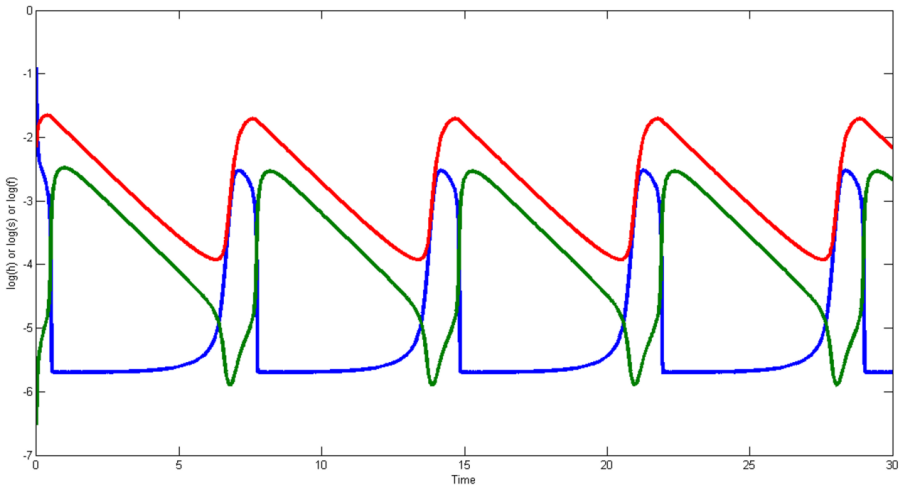


Figure 22 Simulation of the diseased individual with recurrent attacks of inflammation. The constant parameters for the simulation are:
$k_1 = 15.23$, $k_2 = 1.0$, $k_3 = 10^6$, $k_4 = 2.0$, $k_5 = 2000$, $\gamma = 2.0$, $\theta = 0.5$, and $T = 1$.

In our systems we use a set of parameters, which are basically adopted from [79]. This set is reported to be working well in the study of Oregonator system. Other sets can be studied but this is not in the scope of our study except for the analyses done and given in Figures 17 & 18. We showed how clinical features of the population can be explained by the critical rate constants of our models. We showed how people with HLA-B51 can be

categorized into carrier, potent patient, and diseased modes only by changing the values of T and k_1. A study and categorization considering the effect of k_3 can also be done, which would yield information on how the presence of HLA-B51 affects the individual's advantage or disadvantage against diseases targeting the interactions of T-cells with the antigen presenting and infected cells.

One thing to note here is that if two simulations are done with the same constants and initial conditions, the healthy model will clear more of the pathogens (the variable (h) will approach zero more) as opposed to the diseased system [data not shown]. This fact is predictable, since the diseased system will have flares of attacks because of not cleared pathogens. Another note on the triggers (pathogens and self-antigens) is that their amount is taken constant, such that the exposure to these triggers is constant but the amount of infected cells (h) will change its value. Clinical data, [4], reveals that there are pathogenic (bacterial or viral) elements in the oral flora of the patients but they manifest the symptoms in discrete times.

Aside from the healthy system model, our model of the diseased explains the clinical facts such as there are people with HLA-B51 who are not sick, or the effect of HLA-B51 on BD occurrence is almost 20 % only, [25]. This percentage explains that the occurrence of BD in an individual from a family with a history of HLA-B51 presence is only 20 cases per hundred. Although the gene is conveyed to descendants, the progeny may have an altered value for the parameter k_1, and does not show symptoms of BD even if his/her ancestors are diseased. Thus, we can say the value of k_1 is not limited by genetic factors but is a total, metabolic outcome, which depends in every human, and carrying HLA-B51 is mostly not sufficient to induce a disease.

Finally, we verified that the reduced system in (3) behaves similar to the three-variable model in (1). Figure 23 shows results of comparison of the diseased mode of the three-variable model with the variables of reduced form. In Figure 23, the results for h

and f variables from both the normalized three-variable model and the two-variable reduced model are plotted separetely. The reduced form results deviate from those of three-variable model after the first cycle of immune response. There occurs a lag between the two data sets and the reduced form data follows from behind. However, this lag is not significant in our studies since there are flare/remission cycles and they are autonomous. The variable s, however, is not included in the plots since it becomes an algebraic equation in the two-variable system.

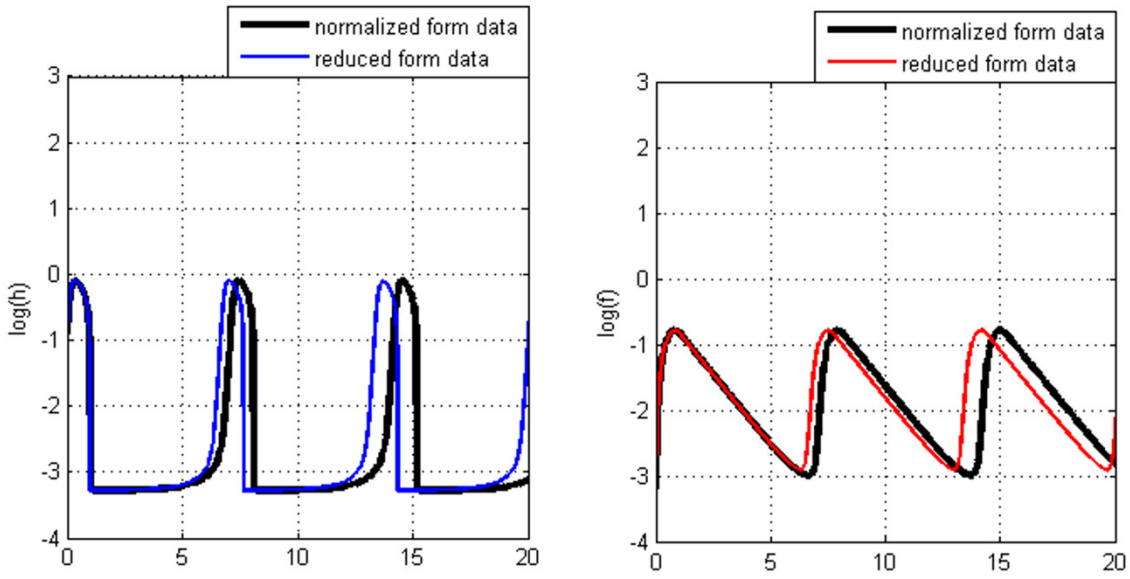


Figure 23 Simulation of the diseased individual (a) with three-variable model and (b) with reduced model.
(a) The parameter values are same with Figure 8. (b) The values of the parameters are: $\varepsilon_1 = 0.06566$, $q = 5.2528 \times 10^{-4}$, $\gamma = 2$, $\theta = 0.5$.

Chapter 5

5. CONCLUSIONS

In this paper, we have proposed, for the first time in the literature, a mathematical model for Behçet's Disease, which is in the form of three coupled nonlinear ordinary differential equations. In accordance with this model, we also proposed a model for the healthy population who do not possess the genetic risk factor of BD, the HLA-B51 allele. These two models differ only in one term for the presence/non-existence of this protein.

The current pathogenesis maps in the literature are updated to yield a new insightful picture. In this study, mathematical models are formulated using the newly formed map and a hypothesis on how BD proceeds. In construction of these models, ordinary differential equations are used. The basic model consists of three state variables representing the HLA-B51, adaptive immune cells, and innate immune cells. Then normalization of the equation system is followed by a reduction maneuver. The information obtained from the bifurcation analysis on the reduced model is used in simulations of the three-variable model. The results of the reduced form and the normalized system are compared and validated that the approximations are accurate.

Our healthy system always manifests stable steady points, which is used to define different modes of health and is consistent with previous studies. Depending on the initial values of pathogens and the rate constants, the system settles at a fixed point, which is retrieved eventually when a pulse input of triggers is given. The diseased model, on the other hand, introduces different phenotypic modes available in populations. The healthy

HLA-B51 carriers, the potent patients, and the diseased individuals are all describable with our model. A person with the HLA-B51 gene allele and high values of k_1 and k_3 would probably demonstrate the symptoms of Behçet's Disease. For lower values of these rate constants or under fairly low antigenic conditions, one may not manifest the recurrent attacks of inflammation even if carrying HLA-B51.

We have proposed two models for healthy and Behçetegonic individuals, one obtained from the other one by a modification that captures the presence of HLA-B51. Our healthy system model forms an alternative for the current inflammation models and is open to modifications to enhance or differentiate into specific disease models, just as it does in our current study. The model of the diseased, on the other hand, explains the clinical findings published in the literature. Being open to new challenges and modifications, our systems set forth the first mathematical model for Behçet's Disease and will help to understand this complex disorder in a quantitative manner.

6. APPENDIX A

Fixed Point 1:

$$h_{ss} : 0.0$$

$$s_{ss} : 0.0$$

$$f_{ss} : 0.0$$

Fixed Point 2:

$$h_{ss} : \frac{1}{2}(1 - q - \gamma\theta - \sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2})$$

$$s_{ss} : \frac{q + q^2 + 3\gamma q\theta + q\sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2}}{4q}$$

$$f_{ss} : \frac{1}{2}(\theta - q\theta - \gamma\theta^2 - \theta\sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2})$$

Fixed Point 3:

$$h_{ss} : \frac{1}{2}(1 - q - \gamma\theta + \sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2})$$

$$s_{ss} : \frac{q + q^2 + 3\gamma q\theta - q\sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2}}{4q}$$

$$f_{ss} : \frac{1}{2}(\theta - q\theta - \gamma\theta^2 + \theta\sqrt{1 + 2q + q^2 - 2\gamma\theta + 6\gamma q\theta + \gamma^2\theta^2})$$

7. APPENDIX B

B.1. *The RHS of the healthy reduced form and its Jacobian*

$$RHS_{healthy} = \begin{bmatrix} -h^2 + \frac{\gamma f(q-h)}{(q+h)\varepsilon_1} \\ \theta h - f \end{bmatrix}$$

$$J_{healthy} = \begin{bmatrix} -2h - \frac{\gamma f}{(q+h)} - \frac{\gamma f(q-h)}{(q+h)^2} & \frac{\gamma(q-h)}{(q+h)\varepsilon_1} \\ \varepsilon_1 & \\ \theta & -1 \end{bmatrix}$$

B.2. *The RHS of the diseased reduced form and its Jacobian*

$$RHS_{diseased} = \begin{bmatrix} h - h^2 + \frac{\gamma f(q-h)}{(q+h)\varepsilon_1} \\ \theta h - f \end{bmatrix}$$

$$J_{diseased} = \begin{bmatrix} 1 - 2h - \frac{\gamma f}{(q+h)} - \frac{\gamma f(q-h)}{(q+h)^2} & \frac{\gamma(q-h)}{(q+h)\varepsilon_1} \\ \varepsilon_1 & \\ \theta & -1 \end{bmatrix}$$

8. BIBLIOGRAPHY

1. Yazici, H., I. Fresko, and S. Yurdakul, *Behcet's syndrome: disease manifestations, management, and advances in treatment.* Nat Clin Pract Rheumatol, 2007. **3**(3): p. 148-55.
2. Verity, D.H., et al., *Behçet's disease: from Hippocrates to the third millennium.* Br J Ophthalmol, 2003. **87**: p. 1175-1183.
3. Saadoun, D. and B. Wechsler, *Behcet's disease.* Orphanet J Rare Dis, 2012. **7**(1): p. 20.
4. Gul, A., *Behcet's disease: an update on the pathogenesis.* Clin Exp Rheumatol, 2001. **19**(5 Suppl 24): p. S6-12.
5. Remmers, E.F., et al., *Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease.* Nat Genet, 2010. **42**(8): p. 698-702.
6. Cho, S.B., S. Cho, and D. Bang, *New insights in the clinical understanding of Behcet's disease.* Yonsei Med J, 2012. **53**(1): p. 35-42.
7. Galeone, M., et al., *Potential Infectious Etiology of Behcet's Disease.* Patholog Res Int, 2012. **2012**: p. 595380.
8. Kaya, T.I., *Genetics of Behcet's Disease.* Patholog Res Int, 2012. **2012**: p. 912589.
9. Kaneko, F., et al., *The role of streptococcal hypersensitivity in the pathogenesis of Behcet's Disease.* Eur J Dermatol, 2008. **18**(5): p. 489-98.
10. Kulaber, A., et al., *Pro-inflammatory cellular immune response in Behcet's disease.* Rheumatol Int, 2007. **27**(12): p. 1113-8.
11. Tojo, M., et al., *Detection of herpes virus genomes in skin lesions from patients with Behcet's disease and other related inflammatory diseases.* Acta Derm Venereol, 2003. **83**(2): p. 124-7.
12. Ergun, T., et al., *HSP 60 expression in mucocutaneous lesions of Behcet's disease.* J Am Acad Dermatol, 2001. **45**(6): p. 904-9.
13. Kibaroglu, A., et al., *T and NK cell subset changes with microbial extracts and human HSP60-derived peptides in Behcet's disease.* Clin Exp Rheumatol, 2004. **22**(4 Suppl 34): p. S59-63.
14. Stanford, M., et al., *Oral tolerization with peptide 336-351 linked to cholera toxin B subunit in preventing relapses of uveitis in Behcet's disease.* Clin Exp Immunol, 2004. **137**(1): p. 201-8.
15. Hu, W., et al., *Experimental mucosal induction of uveitis with the 60-kDa heat shock protein-derived peptide 336-351.* Eur J Immunol, 1998. **28**(8): p. 2444-55.
16. Gogus, F., et al., *Oxidative burst response to monosodium urate crystals in patients with Behcet's syndrome.* Clin Exp Rheumatol, 2005. **23**(4 Suppl 38): p. S81-5.

17. Cakir, N., et al., *Response to intradermal injection of monosodium urate crystals in Behcet's syndrome.* Ann Rheum Dis, 1991. **50**(9): p. 634-6.
18. Gul, A., et al., *Immunohistology of skin pathergy reaction in Behcet's disease.* Br J Dermatol, 1995. **132**(6): p. 901-7.
19. Mendoza-Pinto, C., et al., *Etiopathogenesis of Behcet's disease.* Autoimmun Rev, 2010. **9**(4): p. 241-5.
20. Pay, S., et al., *Immunopathogenesis of Behcet's disease with special emphasize on the possible role of antigen presenting cells.* Rheumatol Int, 2007. **27**(5): p. 417-24.
21. Direskeneli, H., *Behcet's disease: infectious aetiology, new autoantigens, and HLA-B51.* Ann Rheum Dis, 2001. **60**(11): p. 996-1002.
22. Aksu, K., et al., *Prevalences of hepatitis A, B, C and E viruses in Behcet's disease.* Rheumatology (Oxford), 1999. **38**(12): p. 1279-81.
23. Tomiyama, R., et al., *Investigation of the association between Toll-like receptor 2 gene polymorphisms and Behçet's disease in Japanese patients.* Human Immunology, 2009. **70**(1): p. 41-44.
24. Hirohata, S. and H. Kikuchi, *Behcet's disease.* Arthritis Res Ther, 2003. **5**(3): p. 139-46.
25. Gul, A., et al., *Evidence for linkage of the HLA-B locus in Behcet's disease, obtained using the transmission disequilibrium test.* Arthritis Rheum, 2001. **44**(1): p. 239-40.
26. Kim, P.S., D. Levy, and P.P. Lee, *Modeling and simulation of the immune system as a self-regulating network.* Methods Enzymol, 2009. **467**: p. 79-109.
27. Hancioglu, B., D. Swigon, and G. Clermont, *A dynamical model of human immune response to influenza A virus infection.* J Theor Biol, 2007. **246**(1): p. 70-86.
28. Perelson, A.S., *Modelling viral and immune system dynamics.* Nat Rev Immunol, 2002. **2**(1): p. 28-36.
29. Anderson, A.R. and V. Quaranta, *Integrative mathematical oncology.* Nat Rev Cancer, 2008. **8**(3): p. 227-34.
30. Reynolds, A., et al., *A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation.* J Theor Biol, 2006. **242**(1): p. 220-36.
31. Maenaka, K., et al., *Nonstandard peptide binding revealed by crystal structures of HLA-B*5101 complexed with HIV immunodominant epitopes.* J Immunol, 2000. **165**(6): p. 3260-7.
32. Mizuki, N., et al., *Association of Major Histocompatibility Complex Class I Chain-Related Gene A and HLA-B Alleles with Behcet's Disease in Turkey.* Jpn J Ophthalmol, 2007. **51**(6): p. 431-6.
33. Mizuki, N., et al., *Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behcet's disease susceptibility loci.* Nat Genet, 2010. **42**(8): p. 703-6.

34. Kurata, R., et al., *TRIM39 and RNF39 are associated with Behcet's disease independently of HLA-B *51 and -A *26.* Biochem Biophys Res Commun, 2010. **401**(4): p. 533-7.
35. Yanagihori, H., et al., *Lack of association of interleukin-12 p40 gene (IL12B) polymorphism with Behcet's disease in the Japanese population.* J Dermatol Sci, 2004. **34**(2): p. 112-4.
36. Kose, O., et al., *Expression of cytokeratins, adhesion and activation molecules in oral ulcers of Behcet's disease.* Clin Exp Dermatol, 2008. **33**(1): p. 62-9.
37. Kalayciyan, A., et al., *Nicotine and biochanin A, but not cigarette smoke, induce anti-inflammatory effects on keratinocytes and endothelial cells in patients with Behcet's disease.* J Invest Dermatol, 2007. **127**(1): p. 81-9.
38. Han, E.C., et al., *Expression of Pro-inflammatory Protein S100A12 (EN-RAGE) in Behcet's Disease and Its Association with Disease Activity: A Pilot Study.* Ann Dermatol, 2011. **23**(3): p. 313-20.
39. Yasuoka, H., et al., *Autoreactive CD8+ cytotoxic T lymphocytes to major histocompatibility complex class I chain-related gene A in patients with Behcet's disease.* Arthritis Rheum, 2004. **50**(11): p. 3658-62.
40. Zouboulis, C.C., *HLA-independent antibacterial host response toward Th1 immunity mediated by IL-12: a new concept for the pathogenesis of Adamantiades-Behcet's disease.* J Invest Dermatol, 2006. **126**(7): p. 1444-7.
41. Mizuki, N., et al., *Microsatellite mapping of a susceptible locus within the HLA region for Behcet's disease using Jordanian patients.* Hum Immunol, 2001. **62**(2): p. 186-90.
42. Baud, V. and M. Karin, *Signal transduction by tumor necrosis factor and its relatives.* Trends Cell Biol, 2001. **11**(9): p. 372-7.
43. Williams, L.M., et al., *Interleukin-10 suppression of myeloid cell activation--a continuing puzzle.* Immunology, 2004. **113**(3): p. 281-92.
44. Kotenko, S.V., et al., *Identification and functional characterization of a second chain of the interleukin-10 receptor complex.* EMBO J, 1997. **16**(19): p. 5894-903.
45. Ramana, C.V., et al., *Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling.* Trends Immunol, 2002. **23**(2): p. 96-101.
46. Murray, P.J., *The JAK-STAT signaling pathway: input and output integration.* J Immunol, 2007. **178**(5): p. 2623-9.
47. Zhou, J.H., et al., *IL-10 inhibits apoptosis of promyeloid cells by activating insulin receptor substrate-2 and phosphatidylinositol 3'-kinase.* J Immunol, 2001. **167**(8): p. 4436-42.
48. Crawley, J.B., et al., *Interleukin-10 stimulation of phosphatidylinositol 3-kinase and p70 S6 kinase is required for the proliferative but not the antiinflammatory effects of the cytokine.* J Biol Chem, 1996. **271**(27): p. 16357-62.

49. Watford, W.T., et al., *Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4.* Immunol Rev, 2004. **202**: p. 139-56.
50. Nakahira, M., et al., *An absolute requirement for STAT4 and a role for IFN-gamma as an amplifying factor in IL-12 induction of the functional IL-18 receptor complex.* J Immunol, 2001. **167**(3): p. 1306-12.
51. Yu, Q., V.T. Thieu, and M.H. Kaplan, *Stat4 limits DNA methyltransferase recruitment and DNA methylation of the IL-18Ralpha gene during Th1 differentiation.* EMBO J, 2007. **26**(8): p. 2052-60.
52. Becskei, A. and M.J. Grusby, *Contribution of IL-12R mediated feedback loop to Th1 cell differentiation.* FEBS Lett, 2007. **581**(27): p. 5199-206.
53. Yamamoto, K., et al., *SOCS-3 inhibits IL-12-induced STAT4 activation by binding through its SH2 domain to the STAT4 docking site in the IL-12 receptor beta2 subunit.* Biochem Biophys Res Commun, 2003. **310**(4): p. 1188-93.
54. Takatori, H., et al., *Stat5a inhibits IL-12-induced Th1 cell differentiation through the induction of suppressor of cytokine signaling 3 expression.* J Immunol, 2005. **174**(7): p. 4105-12.
55. Wesche, H., et al., *The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases).* J Biol Chem, 1997. **272**(12): p. 7727-31.
56. Dinarello, C.A., *Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process.* Am J Clin Nutr, 2006. **83**(2): p. 447S-455S.
57. Subramaniam, S., C. Stansberg, and C. Cunningham, *The interleukin 1 receptor family.* Dev Comp Immunol, 2004. **28**(5): p. 415-28.
58. Dunne, A. and L.A. O'Neill, *The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense.* Sci STKE, 2003. **2003**(171): p. re3.
59. Bowie, A. and L.A. O'Neill, *The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products.* J Leukoc Biol, 2000. **67**(4): p. 508-14.
60. Kuldo, J.M., et al., *Differential effects of NF-{kappa}B and p38 MAPK inhibitors and combinations thereof on TNF-{alpha}- and IL-1{beta}-induced proinflammatory status of endothelial cells in vitro.* Am J Physiol Cell Physiol, 2005. **289**(5): p. C1229-39.
61. Suzuki, M., et al., *The role of p38 mitogen-activated protein kinase in IL-6 and IL-8 production from the TNF-alpha- or IL-1beta-stimulated rheumatoid synovial fibroblasts.* FEBS Lett, 2000. **465**(1): p. 23-7.

62. Werman, A., et al., *The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription.* Proc Natl Acad Sci U S A, 2004. **101**(8): p. 2434-9.
63. Sada, T., et al., *Association analysis of Toll-like receptor 7 gene polymorphisms and Behcet's disease in Japanese patients.* Hum Immunol, 2011. **72**(3): p. 269-72.
64. Chen, X., et al., *Single nucleotide polymorphisms of Ficolin 2 gene in Behcet's disease.* J Dermatol Sci, 2006. **43**(3): p. 201-5.
65. Wang, H., et al., *Mannose-binding lectin polymorphisms in patients with Behcet's disease.* J Dermatol Sci, 2004. **36**(2): p. 115-7.
66. Ahn, J.K., et al., *CD8brightCD56+ T cells are cytotoxic effectors in patients with active Behcet's uveitis.* J Immunol, 2005. **175**(9): p. 6133-42.
67. Eksioglu-Demiralp, E., et al., *Neutrophil activation in Behcet's disease.* Clin Exp Rheumatol, 2001. **19**(5 Suppl 24): p. S19-24.
68. Poulter, L.W. and T. Lehner, *Immunohistology of oral lesions from patients with recurrent oral ulcers and Behcet's syndrome.* Clin Exp Immunol, 1989. **78**(2): p. 189-95.
69. Sahin, S., et al., *Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behcet's disease.* Ann Rheum Dis, 1996. **55**(2): p. 128-33.
70. Mege, J.L., et al., *Overproduction of monocyte derived tumor necrosis factor alpha, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behcet's disease. A comparative study with familial Mediterranean fever and healthy subjects.* J Rheumatol, 1993. **20**(9): p. 1544-9.
71. Sahin, S., et al., *Monocyte activity in Behcet's disease.* Br J Rheumatol, 1996. **35**(5): p. 424-9.
72. Yamaguchi, Y., et al., *Natural killer cells control a T-helper 1 response in patients with Behcet's disease.* Arthritis Res Ther, 2010. **12**(3): p. R80.
73. Saruhan-Direskeneli, G., et al., *Expression of KIR and C-type lectin receptors in Behcet's disease.* Rheumatology (Oxford), 2004. **43**(4): p. 423-7.
74. Nagafuchi, H., et al., *Excessive expression of Txk, a member of the Tec family of tyrosine kinases, contributes to excessive Th1 cytokine production by T lymphocytes in patients with Behcet's disease.* Clin Exp Immunol, 2005. **139**(2): p. 363-70.
75. Dinarello, C.A., *Proinflammatory cytokines.* Chest, 2000. **118**(2): p. 503-8.
76. Zouboulis, C.C. and T. May, *Pathogenesis of Adamantiades-Behcet's disease.* Med Microbiol Immunol, 2003. **192**(3): p. 149-55.
77. Kanehisa, M. and S. Goto, *KEGG: kyoto encyclopedia of genes and genomes.* Nucleic Acids Res, 2000. **28**(1): p. 27-30.
78. Krummel, M.F., et al., *Differential clustering of CD4 and CD3zeta during T cell recognition.* Science, 2000. **289**(5483): p. 1349-52.

79. Mazzotti, M., M. Morbidelli, and G. Serravalle, *Bifurcation Analysis of the Oregonator Model in the 3-D Space Bromate/Malonic Acid/Stoichiometric Coefficient.* J. Phys. Chem., 1995. **99**(13): p. 4501-4511.

VITA

Cemal was born in 1988 in Reşadiye in Tokat. In 2010, he graduated from METU Chemical Engineering Department. In late 2010, he joined Erman Research Group, which is renamed as Computational and Quantitative Biology Lab. He was both a research and teaching assistant in Koç University.