Cardiac antigen derived T cell epitopes in the frame of myocardial infarction

-

T-Zell-Epitope von kardialen Antigenen im Kontext des Myokardinfarktes

Doctoral thesis for a medical doctoral degree
at the Graduate School of Life Sciences,
Julius-Maximilians-Universität Würzburg,
Section Biomedicine

submitted by

Nils Hapke

from

Hannover

Würzburg 2022

Submitted on:

Members of the Thesis Committee:

Chairperson:
Prof. Lars Dölken

Primary Supervisor:
Prof. Dr. Stefan Frantz

Supervisor (Second):
Dr. Gustavo Ramos

Supervisor (Third):
Prof. Dr. Andreas Beilhack

Supervisor (Fourth):
Prof. Dr. Thomas Kerkau

Date of Public Defence:

Date of Receipt of Certificates:

The doctoral candidate is an approbated physician.

Table of contents

Table of figures

Zusammenfassung

Die koronare Herzerkrankung und die akute Konsequenz des Myokardinfarktes (MI) sind eine der häufigsten Ursachen von Morbidität und Mortalität in unserer westlichen Gesellschaft. Obwohl es große Fortschritte in der Behandlung von akut lebensbedrohlichen ischämischen Ereignissen gab, bleibt die resultierende Herzinsuffizienz nach Infarkt ein häufiges klinisches Problem. Immer mehr Evidenz weist auf eine wichtige Rolle von T-Zellen im Heilungsprozess nach MI hin, aber relevante Autoantigene, die adaptive Immunantworten auslösen und regulieren könnten, wurden in Patienten mit MI noch nicht entdeckt.

In dieser Arbeit beschreiben wir ein Epitop des Adrenergen Rezeptors Beta 1, der im Herz hoch exprimiert ist und als Autoantigen fungiert. Dieses Autoantigen verursacht eine pro-inflammatorische Immunreaktion in T-Zellen, die von MI-Patienten isoliert wurden, aber nicht in Kontrollpatienten. Diese Immunreaktion beobchten wir jedoch nur in einem Teil der Patienten, der ein Allel der Familie HLA-DRB1*13 trägt. Interessanterweise sind MI-Patienten häufiger Träger eines solchen Allels als Kontroll-Patienten.

Zusammenfassend legen unsere Ergebnisse nahe, dass T-Zellen in MI-Patienten antigen-spezifisch aktiviert werden und einen pro-inflammatorischen Phänotyp ausbilden. Die aktivierten T-Zellen reagieren ex vivo auf ein kardiales Autoantigen und entwickeln vermutlich in vivo einen ähnlichen Phänotyp. Dieser ist abhängig von einem HLA-Allel, welches in Infarkt-Patienten häufiger war als in der Kontrollgruppe, was eine mögliche Rolle als Risikofaktor für kardiovaskuläre Erkrankungen suggeriert.

Unsere Ergebnisse stellen eine wichtige Grundlage dar, um unser Verständnis des Immunsystems in kardiovaskulären Erkrankungen zu vertiefen. Wir beschreiben in dieser Arbeit das erste kardiale Autoantigen, das im klinischen Kontext des Myokardinfarktes entdeckt wurde und bieten somit eine wichtige Grundlage für weitere translationale und klinische Forschung in der Immunkardiologie.

Summary

Cardiovascular disease and the acute consequence of myocardial infarction remain one of the most important causes of morbidity and mortality in all western societies. While much progress has been made in mitigating the acute, life-threatening ischemia caused by infarction, heart failure of the damaged myocardium remains prevalent. There is mounting evidence for the role of T cells in the healing process after myocardial infarction, but relevant autoantigens, which might trigger and regulate adaptive immune involvement have not been discovered in patients.

In this work, we discovered an autoantigenic epitope in the adrenergic receptor beta 1, which is highly expressed in the heart. This autoantigenic epitope causes a pro-inflammatory immune reaction in T cells isolated from patients after myocardial infarction (MI) but not in control patients. This immune reaction was only observed in a subset of MI patients, which carry at least one allele of the HLA-DRB1*13 family. Interestingly, HLA-DRB1*13 was more commonly expressed in patients in the MI group than in the control group.

Taken together, our data suggests antigen-specific priming of T cells in MI patients, which leads to a pro-inflammatory phenotype. The primed T cells react to a cardiac derived autoantigen ex vivo and are likely to exhibit a similar phenotype in vivo. This immune phenotype was only observed in a certain subset of patients sharing a common HLA-allele, which was more commonly expressed in MI patients, suggesting a possible role as a risk factor for cardiovascular disease.

While our results are observational and do not have enough power to show strong clinical associations, our discoveries provide an essential tool to further our understanding of involvement of the immune system in cardiovascular disease. We describe the first cardiac autoantigen in the clinical context of MI and provide an important basis for further translational and clinical research in cardiac autoimmunity.

1 Introduction

Over the many, many years of evolution, we adapted very well to most of the dangers and inconveniences, that life confronts us with. From the bacteria and viruses our organism defends against to the microbiome we tolerate, our immune system has become remarkably good at differentiating bad from good and dangerous from beneficial. While this sounds easy, when we talk about pathogens, it becomes much more complicated to wrap your head around the mechanisms that evolved to defend ourselves against imbalances that occur without the direct involvement of non-self.

In our modern society, infectious diseases are comparably well controlled and are not the biggest killers in the developed world anymore. On the contrary, non-communicable diseases become very important as we grow older than ever before and adapted a sedentary lifestyle in nutritional abundance. Aging leads to atherosclerosis and one of the biggest killers of our modern age – myocardial infarction.

This work tries to provide some interesting ideas on the relevance of the immune system in a society that is more endangered by non-communicable diseases than ever. While the role of the immune system is not very well understood in this setting, it becomes ever more important to develop insights and a deeper understanding. We aim to facilitate further research by exploring immunity directed against self in the context of myocardial infarction, as an example of dynamic tolerance – the tolerance of the immune system against self, with a temporary autoimmune reaction in case of injury.

1.1 Complementary arms of the immune system

The immune system is traditionally divided into the innate and the adaptive immune system each including various cell types. Both have intertwined functions and often work in synergy. While there are multiple ways in which the immune system recognizes pathogens, one important concept is that of an antigen – a certain peptide which induces an immune reaction.

As this work focuses on the relevance of antigens derived from self, it is important to understand mechanisms of antigen recognition and how these prevent and sometimes allow recognition of self.

The main distinction of adaptive and innate cells is that adaptive cells develop antigen-specific receptors, that are not germline-encoded in the DNA and thus inherited but developed by somatic rearrangement during immune cell development. Every adaptive immune cell thus develops a unique receptor, generating a large repertoire of antigen-recognizing receptors. Innate immune cells on the other hand express receptors, which are inherited and similar between cells.[1]

1.1.1 Innate immunity

It was discovered that innate immune cells possess a repertoire of receptors – termed Toll-like-receptors (TLRs) – which can recognize common pathogen associated molecular patterns (PAMPs) and initiate an inflammatory response. This recognition enables a general differentiation of self and non-self with limited pathogen-specificity. TLRs are germline-encoded in the DNA, so there is no variation of this receptor among expressing cells, but different types of TLRs exist.[2] Importantly, so called damage-associated molecular patterns (DAMPs) were discovered, which comprise of motifs exposed after cellular injury and which lead to an inflammatory reaction in a similar fashion as PAMPs.[3]

Most innate immune cells are so called myeloid cells. Myeloid cells are a diverse compartment of leukocytes that originate in the bone marrow and all originate from a progenitor cell called “myeloblast”.[4] With the advancing popularity of scRNA-sequencing technologies, an impressive heterogeneity of these

cell subsets is becoming obvious, including various subsets of monocytes, macrophages and dendritic cells.[5] As numerous as these cells are, is their function. Regarding antigen-specific immunity, one pivotal function of myeloid cells is the uptake, processing, and presentation of antigens.

One prominent type of myeloid cells are the macrophages, which are very efficient phagocytic cells. While these cells are important for removing necrotic debris, the most prominenT cells for activation of T cells are the dendritic cells (DCs).[1] As a big part of immune activation happens in lymph nodes, there need to be phagocytic cells, that can take up cell debris and transport it to the lymph nodes and DCs efficiently fulfil this role. [1]

Activated dendritic cells migrate to the lymph nodes and come into contact with T cells. Depending on their prior activation, the dendritic cells express a different set of surface molecules and secrete varying cytokines, influencing the polarization of T cells.[6] T cells that recognize peptides presented by these activated dendritic cells are activated and expand. T cell development and activation will be described later in further detail (→T cells).

In the steady state, DCs are important to maintain tolerance and prevent autoimmunity.[7] However, DCs can have an opposing effect and favor autoimmunity in a pro-inflammatory setting with TLR stimulation.[8] Indeed, different DC subsets were shown to influence autoimmunity after MI.[9]

1.1.2 Adaptive immunity

The most prominent adaptive immune cells are B Cells and T cells, both bearing antigen-specific receptors, which are not germline-encoded, but developed by so called somatic recombination. B Cells are important for producing antibodies, which are developed from the B Cell receptor (BCR). While development of this receptor is in large parts equivalent to the T cell equivalent (TCR), they are of minor importance for this work.[1]

T cells are a type of antigen-specific immune cell, but they do not directly bind to antigens, as antibodies do. Instead, a T cell Receptor (TCR) binds specifically to a complex of antigenic peptide and a presenting molecule.[1]

Major histocompatibility complexes (MHC) are special proteins, which can present short antigenic fragments on the cell surface to be recognized by T cells. Different types of MHC can presenT cell-intrinsic antigens as well as extracellular antigens, which are processed after phagocytosis. This enables T cells to not only target soluble, free-floating antigens, but also develop an adaptive immune response to intracellular antigens. These different types of MHC are mainly classified as MHC class I and MHC class II, which differ in the source of antigen they are presenting.[1]

Classical teaching describes the TCR-pMHC interaction as a very specific lock-and-key principle, which does not quite convey the complexity of this special interaction. As Don Mason illustrates, it is necessary for a single TCR to recognize a wide array of pMHC to ensure an efficient immune response.[10] A single autoimmune TCR was described to recognize more than a million different peptides.[11]

T cells are classically divided into $CD4^+$-T cells and $CD8^+$-T cells based on the expression of surface molecules.

CD8 enables T cells to bind to MHC-I-molecules, which are present on all nucleated cells in the human body. This enables CD8 cells to fight intracellular infections or malignancies in virtually all tissues in the body.[1] As we focus on the role of $CD4^+$-T cells, MHC-I is of minor importance for this work.

CD4 enables T cells to bind to MHC-II-molecules, which most commonly present phagocytosed extracellular antigens. $CD4^+$-T cells can then respond in a variety of ways, enable or suppress immune responses or provide signals for other immune cells to alter their behavior. T cells are assumed to be important regulators for the activity of the immune system.[1]

1.1.2.1 MHC of mice and humans

Most of the knowledge about mammalian immune systems was discovered in animal experiments, with mice being the experimental tool of choice in most projects.[12] While there are many similarities of the murine to the human immune system and most insights can be transferred, there are some important differences. This work focuses on *ex vivo* analyses of human samples, so concepts of human adaptive immune responses are important to understand the study. Of special importance is the difference in antigen presenting molecules between lab mice and patients.

The general principles of antigen presentation will be explained in the next chapters (→T cells; →Antigen Processing and Presentation). In this general description, major histocompatibility complex II (MHC II) will be used as an example, which is a type of mouse antigen presenting molecule. While most principles also hold true in human patients, there is an important difference in the genomics, as humans are not isogenic lab animals, but genetically very diverse. MHC molecules are called human leukocyte antigens (HLA) in humans and were shown to have remarkable genetic diversity.[13]

1.1.2.2 HLA heterogeneity

As antigen presentation is of high importance in defending our bodies against disease, there is a constant evolutionary fight between pathogens and the immune system. Likely because of adaptation in this evolutionary fight, MHC genes show a remarkable polymorphism and genetic complexity.

HLA-B, which is a human MHC gene and the most polymorphic one known, has at least 2,000 known alleles.[13] It was first suggested more than 70 years ago, that this remarkable polymorphism might be driven by infectious diseases.[14] As our adaptive immune system depends on antigen recognition to effectively defend ourselves against pathogens, it is only natural that most of this heterogeneity focuses on the peptide-binding groove of HLA molecules, thus

influencing the presented antigenic peptides. It has been shown, that HLA heterogeneity also influences mating preferences, offering an explanation for the evolutionary upkeep of HLA-variety.[15] A patient population can be expected to show a diverse variety of HLA genotypes, which influence susceptibility against autoimmune disease and cancer, suggesting an importance for presentation of autoantigens.[16,17]

This remarkable complexity makes the prediction of autoantigens very difficult.

1.1.3 T cells

1.1.3.1 Development

To understand the diverse repertoire of TCRs and the arising possibility of antigen-recognition and immune responses, a short overview of T cell development is helpful. In short, there are two main difficulties in generating a functional TCR-repertoire. First, the antigenic diversity, the organism could potentially be confronted with, is very large and it is not feasible to encode all necessary TCRs in the human genome. Second, it is essential to select a repertoire with broad reactivity to pathogens but prevent autoreactivity and autoimmune disease. How this is accomplished is shortly described in the following.

T cell progenitors originate from the bone marrow. After migration of bone marrow stem cells into the thymus, the main organ of T cell development, commitment to the T cell lineage is mediated by the microenvironment.[18] During cell development in the thymus, genes for the TCR are rearranged and random nucleotides are inserted, thus generating a unique receptor for every progenitor cell. This process is called somatic recombination.[1]

As the generated TCRs are partly random, the repertoire covers a vast range of antigens, but could also include dysfunctional or self-reactive T cells.

To prevent circulation of these, there is a further selection in the thymus.[1] T cells are tested for reactivity to self-antigens presented on MHC (self-MHC), which are expressed on the surface of thymic cells. Very low reactivity to self-MHC leads to death by neglect and apoptosis of T cells, as these cells would be likely dysfunctional. Thus, only functional T cells are selected in a process called positive selection. High reactivity to self-MHC leads to elimination to prevent self-reactive T cells from entering circulation and potentially causing autoimmune disease. This process is called negative selection.[19]

However, some T cells with intermediate affinity to self-MHC are not deleted but commit to a special phenotype termed "regulatory T cell" (Treg).[1] These Tregs are regulators of the immune response and suppress immune cell activation. As they prevent overactivation, they are essential for preventing autoimmune disease.

Treg cells created this way in the thymus are termed natural Tregs (nTregs).

The final repertoire of T cells entering circulation is depleted of highly self-reactive cells, but still encompasses TCRs with affinity for self. How the immune system maintains a stable balance and sustains self-tolerance, will be explored in further detail later in this work (→ Self-Tolerance).

1.1.3.2 Priming and Activation

After development in the thymus, T cells enter circulation and comprise the repertoire of "naïve" T cells. While already bearing a mature TCR, these cells have not yet encountered any antigen-specific stimulation. To induce activation and proliferation and a functional immune response, naïve T cells need to be primed by special antigen presenting cells, the dendritic cells.[1,6]

To assure a potent immune response, even to antigens that the immune system has never encountered before, it is important for the naïve T cells to constantly

circulate through the body. In the secondary lymphoid organs (lymph nodes, spleen, tonsils), they encounter antigen-presenting cells such as dendritic cells. Through this constant circulation, even rare T cell clones are able to mount an immune response, should the body encounter their specific antigen.[20]

1.1.3.3 Phenotype and function

Activated T cells can differentiate into diverse phenotypes with different functions. The phenotype of the T cell response is an important mediator of immune function.[1] As T cells play an important role in coordinating immune responses, the proper differentiation needs to be tightly controlled to prevent autoimmune reactions as well as insufficient immune responses. *In vitro* evidence suggests an important role of cytokines in determining the phenotypic differentiation of naïve T cells.[21]

The so called “conventional” T cells (Tconv), which exclude Tregs, were initially separated into two subsets, Th1 and Th2, with different cytokine signatures and cell surface molecules.

Th1 cells mainly produce IFN-γ and lymphotoxin, signifying their proinflammatory capacity. They also tend to be good producers of IL-2.[21] Th1 cells are classically thought of as pro-inflammatory cells that help in fighting intracellular infections.[1]

Th2 cells’ signature cytokines are IL-4, IL-5 and IL-13 and they can produce modest amounts of IL-2. Their most prominent function is the coordination of immune responses against extracellular parasites.[1]

A third major effector population, Th17 cells, was discovered some years after, challenging the dichotomy of Th-phenotypes. Th17 cells are characterized by the production of IL-17A, IL-17F and IL-22 and activate neutrophils in clearing extracellular pathogens.[1]

In addition to previously known nTregs, it was also discovered that naïve T cells are able to differentiate into a subset with similar characteristics, termed induced Tregs (iTregs).[21]

T cells of the four described phenotypes typically migrate to and enter the tissues to mediate their immune functions directly. However, another important role of T helper cells is mediation of B cell responses, which happens mainly in lymph nodes and the spleen.

Recently, B follicular helper T cells (Tfh) have been discovered as an independent subset with importance in B cell priming and antibody production.[22] There is an ongoing discussion about the nature of Tfh and whether they might constitute only an activation state within an effector program. In this model, Tfh and effector T cells (Teff) would present different activation states of the described T cell phenotypes.[23]

Although T cell functions are numerous and not very well understood, their relevance for immune responses is very clear. Which phenotype they mount in which antigen-specific subset largely determines the immune effects. Although cytokines as triggers for T cell commitment to certain phenotypes have been identified, which circumstances determine T cells with certain specificities to differentiate remains enigmatic.

As N.K. Jerne put it:[24]

"*Many things are required for the grass to grow, but they don't determine the kind of grass.*"

1.1.4 Antigen Processing and Presentation

1.1.4.1 MHC Antigen Presentation

As the two most common types of T cells are classified as $CD4^+$ and $CD8^+$, the respective antigen-presenting molecules are classified as MHC-II (recognized by $CD4^+$-T cells) and MHC-I (recognized by $CD8^+$-T cells).[1]

The pathway of antigen processing for the different MHC-molecules differs and leads to a divergent antigen repertoire. MHC-I presents mostly antigens from the endocytic pathway, consisting of peptides produced in the cell itself. MHC-I is present on all nucleated cells, presenting an opportunity to survey the proteasome and keep viral infections or tumors in check.[1]

The peptides presented on MHC-II are mostly processed in the exogenous pathway and derived from phagocytosis. MHC-II is mostly expressed by cells of the immune system and aids in coordination of the immune response.[1]

As MHC-II-restricted peptides are mostly derived from extracellular antigens, antigen-presenting cells need to have some capacity to take up and process extracellular material. We are interested in autoreactivity against cardiac antigens. As we assume these antigens to be derived from necrotic cell debris, we focus on MHC-II presentation of cardiac antigens.

To understand the diversity of $CD4^+$ -T cells, it is important to have a basic understanding of the development of MHC-II and the antigen repertoire that is presented on them.

1.1.4.2 MHC-II maturation

To ensure proper peptide binding and presentation, MHC-II undergoes an extensive maturation process before being expressed on the cell surface.

After translation, the α- and β-subunit of MHC-II are associated with the help of a chaperone called the “invariant chain” (I chain) in the endoplasmatic reticulum. The I chain is suggested to help stabilize the MHC-II-complex and prevent antigen-binding in the early phase of MHC-II assembly. Furthermore, the I chain aids with translocation to the endocytic pathway, where processed antigens can be bound to the MHC-complex.[25]

I chain is then progressively degraded in acidic endosomes until a short peptide of about 20 amino acids length remains, called the class II–associated invariant chain peptide (CLIP)[26,27]. As CLIP blocks the peptide binding pocket of MHC-II, it is suggested to play a role in preventing autoimmunity and regulate presentation of self-peptides.[28]

To enable peptide binding to MHC-II in late endosomes, CLIP needs to be dissociated from the complex. This is facilitated by a glycoprotein called DM, which shows high homology to MHC-II. The acidic pH, which is present in late endosomes, facilitates this dissociation, and enables peptide binding to the MHC-II-complex.[29-31]

1.1.4.3 Antigen uptake and processing

As presented antigens are bound in endosomes but are of mostly extracellular origin, they first need to be taken up and processed by antigen presenting cells (APCs). APCs have different routes of antigen uptake, which are mostly mediated by surface receptors. These include receptors such as the C-type lectin family receptor DNGR-1, which promotes antigen presentation from phagocytosed necrotic cells.[32]

After phagocytosis, antigens need to be processed, as MHC-molecules only present short peptides and not whole proteins. However, it is disputed whether processing is necessary before presentation or whether MHC-binding of large molecules precedes subsequent trimming by proteases.[33]

Processing happens in endosomes, which mature from early endosomes into late endosomes and lysosomes. During maturation, the pH of the endosomes acidifies, influencing the activity of different resident proteases, which cleave and process antigens. [32]

A diverse repertoire of proteases, such as cathepsins, is necessary for antigen processing. However, different APCs express different proteases, signifying a different role in antigen presentation.[32] Protease expression is further influenced by stimuli, such as TLR-signaling, influencing the efficiency of antigen presentation.[34]

1.1.4.4 Antigen repertoire

Contrary to MHC-I molecules, which have a closed end peptide-binding cleft, MHC-II molecules allow binding of larger peptides – as long as an epitope of about 9-15 amino acids settles into the binding groove. Experimental evidence shows an average length of 13-22 amino acids for eluted MHC-II peptides.[33]

Affinity to MHC molecules is important for antigen presentation, but not all peptides with a high affinity for MHC induce a response when provided in the context of a whole protein antigen.

"Immunodominance" describes the phenomenon, that a majority of responding $CD8^+$-T cells often only target a few antigenic epitopes following an infection. Although there are multiple possible MHC-alleles for antigen presentation, that could in theory present hundreds of antigens, a large portion of reactive T cells focus on few epitopes, termed "immunodominant epitopes".[35]

This immunogenicity is not simply determined by MHC-binding affinity, but availability for binding of certain protein regions also play a role, making it difficult to accurately predict antigenic epitopes.[36] Interestingly, high affinity peptides competitively inhibit binding of other peptides to MHC molecules, favoring some "immunodominant" epitopes to induce an immune response.[33]

1.2 Self-Tolerance

One might find the idea to look for T cells directed against self-epitopes strange, as one classical concept of immunology is the establishment of tolerance by deletion of self-reactive T cells in the thymus. However, this simple view of complete clonal selection and deletion was challenged often and from the start.[37-39]

As described (→ Development), generation of the TCR is at least partially random to generate a repertoire that can mount responses against all possible pathogens. This works well for defense against infectious disease but makes the process of self-tolerance more difficult. A totally random repertoire would naturally also target self-antigens and lead to autoimmune disease. As the adaptive immune system is also needed to prevent development of cancer and aid in wound healing, complete ignorance of self-antigens would not be beneficial. This makes a delicate balance of immune function necessary that does not lead to autoimmune disease but is still able to have sufficient effector functions against self-antigens.

To unite the necessities of a functioning immune system under one model, it would not be enough to simply "delete" clones of T cells that are directed against self-antigens. It would sometimes be necessary for immune cells to mount a response against self to avert danger. The "Danger Model" elegantly integrates these ideas into a model to explain the balance of activity and tolerance of immune responses. In essence, the immune system might not differentiate between "self" and "non-self", but between "no-danger" and "danger".[40,41]

An interesting example for the delicate balance is the antigen-presenting molecule HLA-B27. This certain MHC-allele is usually discussed as a predisposing factor for the development of various autoimmune diseases. It is, however, not uncommon, which suggests some kind of selective advantage in evolutionary selection to preserve this allele. One suggested advantage is the better

presentation of viral antigens, which improves the immune response against certain pathogens. This might have been an advantage in our evolutionary past and the efficient antigen presentation might have led to efficient presentation of self-antigens as a side effect.[42] This suggests shared presentation mechanisms of self- and foreign antigens and opens the possibility for a similar effect allowing cardiac autoimmunity.

In summary, the human immune system developed many delicately balanced mechanisms to ensure tolerance against self and prevent autoimmune reactions. To sustain the evolutionary pressures of the past, it was probably more important to mount an efficient defense against pathogens, neglecting the danger of collateral immune damage against self. However, it would be wrong to assume that self-tolerance means complete self-ignorance and there are numerous examples of well-balanced autoimmune reactions.

1.3 Tissue Injury and Wound Healing

One such autoimmune reaction can be observed in the process of wound healing. Besides the biological dangers of pathogens and cancer, there are also simple mechanical dangers our body is confronted with. From injuries such as broken bones or injured skin to very complex ischemic wounds after myocardial infarction, there are many examples of injury after which the organism must deal with dead tissue. This tissue is usually not functional anymore and has to be replaced by new functional tissue or a scar. The process of removing debris and replacing it with new tissue is immunologically very interesting, because there needs to be an acute inflammatory reaction against the body's own tissues in a sterile environment. This makes some self-reactivity of the immune system necessary to clear necrotic debris and restore the wounded tissue. To prevent bystander damage, this self-reactivity needs to be tightly controlled and short-lived.

Like the recognition of PAMPs by TLRs, DAMPs are danger associated molecular patterns that aid in recognition of damage to cells. This recognition is suggested to be directed against “hidden self”, some motives that are usually hidden inside cells and are only exposed upon uncoordinated cell disintegration.[43]

Recognition causes an activation of immunity upon cell injury, that is similar to a reaction against pathogens and falls in line with the previously described “danger model”.[40] Dendritic cells could be activated when they sense danger, and in turn provide costimulatory signals to T cells in addition to presentation of antigenic peptides. This would induce a T cell response against self-antigen derived from necrotic cell debris.[43] This train of thought leads to the assumption that autoreactive T cells might be expanded after MI, a main question we set out to investigate in this work.

1.4 Myocardial infarction as a model for Wound Healing

1.4.1 Myocardial Infarction

Ischemic heart disease with the most acute exacerbation of myocardial infarction (MI) remains the top killer of our modern age.[44] The rise of interventional cardiology brought huge improvements to care for patient with acute myocardial infarction, but there remains a high burden of morbidity. The cause and treatment of ischemia is well understood, and blocked arteries can be reopened. How the body deals with the subsequent tissue damage remains more enigmatic.

While the involvement of the immune system in a non-communicable disease such as cardiovascular disease has traditionally not been obvious, recent research demonstrates a remarkable impact of the immune system in practically all stages of the disease. As the field of immunocardiology is growing rapidly, I will focus on the most relevant aspects for this present work. Other excellent

reviews show the extensive involvement of the immune system in cardiovascular disease.[45,46]

1.4.2 Immune System in Myocardial Infarction

To develop better diagnostic and therapeutic tools, it is vital to understand the immune reactions following ischemic injury of the heart as well as their interplay with atherosclerosis, which initially leads to ischemic heart disease. It was shown that these processes are not a simple sequence but are connected, as atherosclerosis accelerates after MI.[47]

It is our goal to shed some light on the immune changes and T cell reactions in the days following MI. There is an interesting body of research investigating the immune system after MI, but the paramount role of T cells is only starting to get appreciated.

1.4.2.1 Innate Immunity

Shortly after the heart becomes ischemic, innate immune cells start to respond by releasing pro-inflammatory mediators. This induces the production and recruitment of neutrophils and monocytes to the injured myocardium. These are mainly recruited from the bone marrow, but the spleen also contributes as a monocyte reservoir.[48,49]

In a first wave, these recruited cells contribute to the immune response in the myocardium for the first few days following MI.

After several weeks, immune cell populations in the heart decrease again and leave behind a durable scar. It is vital for the immune reaction to be in the right balance as too much or too little inflammation leads to a suboptimal healing process.[45]

1.4.2.2 Adaptive Immunity

In addition to innate immune cells, it was also shown that T and B Cells accumulate in the heart after MI. Recruited T cells include mostly Th1 and Treg cells.[50] Recruitment to the site of injury suggests a role of these adaptive immune cells in the wound healing process.

Indeed, T cells were shown to proliferate in heart draining lymph nodes and markedly impact the healing process after MI.[46,51] Tregs were shown to improve healing after MI in mice and depletion was associated with aggravated cardiac inflammation. These changes are likely caused by modulation of monocyte/macrophage differentiation, which suggests the complex innate immune reactions to be orchestrated by a similarly complex adaptive immune response.[52]

Experimental evidence suggests that this T cell influence is dependent on antigen-specific recognition by the TCR, which is supported by evidence for the importance of antigen-presenting dendritic cells in this setting.[9,53]

There is data on the relevance of certain epitopes in the mouse model, which were shown to trigger cardioprotective T cell responses after MI.[54] Interestingly, the same epitopes were shown to fuel a detrimental autoimmune myocarditis in a different setting,[55] highlighting the complicated balance and complexity of immune reactions in regard to one's own heart and allowing some first insights into a fascinating process.

However, relevant antigens have not been discovered in the clinical context and the question remains, whether these insights can be translated to the clinic. There is some evidence for T cell responses directed against autoantigens related to artherosclerosis.[56] To better understand the causes and dynamics of the adaptive immune response in humans is essential to develop novel treatment options for myocardial infarction.

To further our knowledge of the dynamic immune mechanisms involved in wound healing after MI, we designed this study. We believe it to be essential to characterize self-antigens and map the dynamic reactivity against these to understand and quantify the trade-off between autoreactivity and tolerance.

1.5 Aims

The healing process after MI can be impacted by adaptive immune responses, but little is known about the exact mechanisms. A central part of adaptive immune regulation are T helper cells and these were shown to be involved in healing after MI.[46,51,54] However, it is not clear whether these T helper cells bear TCRs against a specific cardiac autoantigen, which might induce their expansion, and if so, which are the relevant immunodominant epitopes. We designed this study to investigate the existence of antigen-specific T helper cells in the context of MI and discover immunodominant epitopes derived from cardiac autoantigens.

To test this hypothesis, we specifically set out to answer the following questions:

Can we identify a $CD4^+$-T cell autoantigen of relevance in the context of MI?

Does T cell autoreactivity affect risk for MI or outcome after ischemic events?

Are there subsets of patients with different immune responses and outcomes?

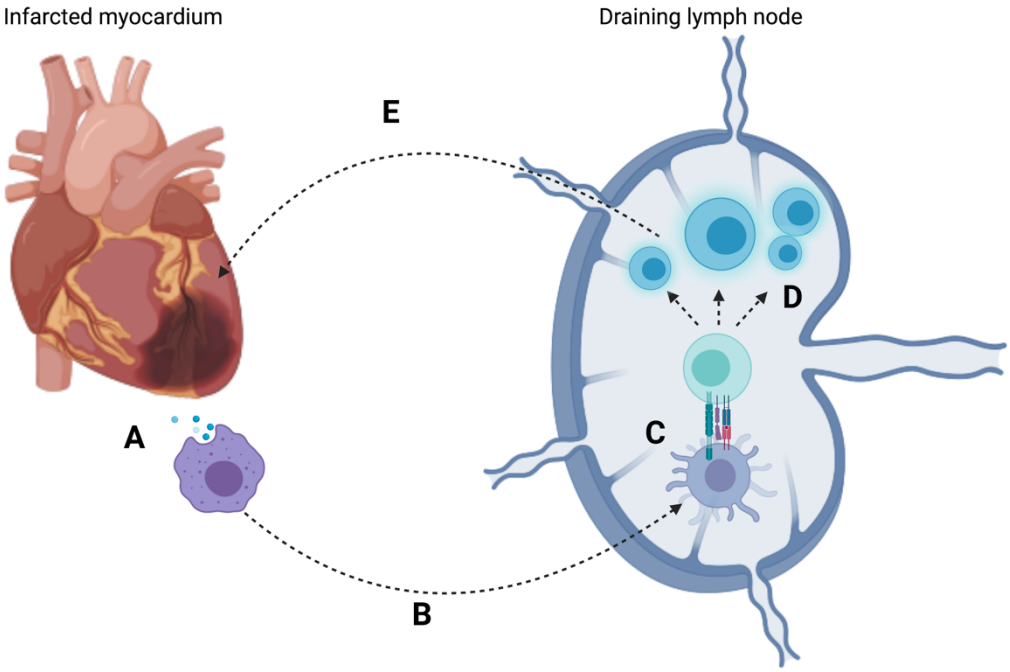

Figure 1 T cell activation after myocardial infarction
(A) Phagocytic cells take up necrotic debris after myocardial infarction and get activated. (B) They then migrate to local draining lymph nodes, where (C) they come into contact with a T cell and present the phagocytoses antigens in a pro-inflammatory context. (D) T cells get activated and proliferate. This causes an auto-antigen-specific pool to expand, which (E) enters circulation and migrates to the heart and provides effector functions. [9,57]

2 Materials and Methods

2.1 Materials

2.1.1 Fine chemicals and reagents

Ethanol	Sigma-Aldrich
2-Propanol	Roth
PBS Dulbecco w/o Ca2+ w/o Mg2+	Biochrom GmbH
Tween 20	Sigma
Nonfat Dry Milk	Cell Signaling Technology
Ethanol	Sigma-Aldrich
DMSO	Sigma
Aqua ad iniectabilia	Braun
Terralin liquid	Schülke & Mayr

2.1.2 Ready for use kits and solutions

Freezing Media Kit (Cryo ABC)	C.T.L.
CTL Wash buffer	C.T.L.
CTL Test	C.T.L.
RPMI	Thermo Fisher
Glutamax	Thermo Fisher
Legendplex Human Th Panel Assay	BioLegend
Elispot Kit	C.T.L.
Human IL-6 ELISA	BioLegend
GeneJET DNA Purification Kit	Thermo Scientific

2.1.3 Biomolecules and compounds

Peptides were custom synthesized by JPT Peptide Technologies GmbH (Berlin) and delivered in a lyophilized state. CEFX-peptides were also ordered from JPT.

2.1.4 Consumables

96-well plates (V-bottom, U-bottom)	Greiner
Nitrile gloves	Hartmann
96-well Maxisorp Nunc-Immuno-plate	Thermo-Fisher
Centrifuge tubes (50ml, 15ml)	Greiner
Parafilm	American National Can
Platesealer Easyseal	Greiner
Pipette tips (20µl, 300µl, 1000µl)	Eppendorf
Filter tips (10µl, 100µl, 200µl, 1000µl)	Biosphere
Pipette (5ml, 10ml, 25ml)	Greiner
Reagent Reservoir (25 ml)	Thermo-Fisher
Cryo tube (1.8ml)	Thermo-Fisher
Combitips (5 ml, 2.5 ml, 1ml)	Eppendorf
BD Vacutainer CPT Tube	BD Biosciences
PCR tubes (0.2 ml)	Eppendorf

2.1.5 Instruments

Analytical balance	Kern
Magnetic stirrer	Ika
Plate shaker	Heidolph
Vortex mixer	Ika
Pipette controller	Brand
Multipette stream	Eppendorf

Multichannel pipette (300 µl)	Eppendorf
Centrifuge 420R	Hettich
Incubator, Hera cell 240i	Thermo-Fisher
Laminar flow hood	Steril S.p.A.
Microscope DMi1	Leica
Centrifuge Mikro 200	Hettich
Cryo 1°C Freezing Container	Nalgene
Neubauer Chamber	Optik Labor
Elispot plate reader	C.T.L.

2.2 Methods

To answer the proposed questions, we followed a two-step approach. First, *in silico* predictions were used to predict likely cardiac epitopes, which might function as autoantigens. These were selected for binding potential to the most relevant antigen presenting molecules in the context of T helper cell reactions.

Second, we designed a clinical study including patients suffering from myocardial infarction and a control group, to collect immune cells. The predicted epitopes were then used to stimulate the immune cells *in vitro* in an attempt to validate our *in silico* predictions.

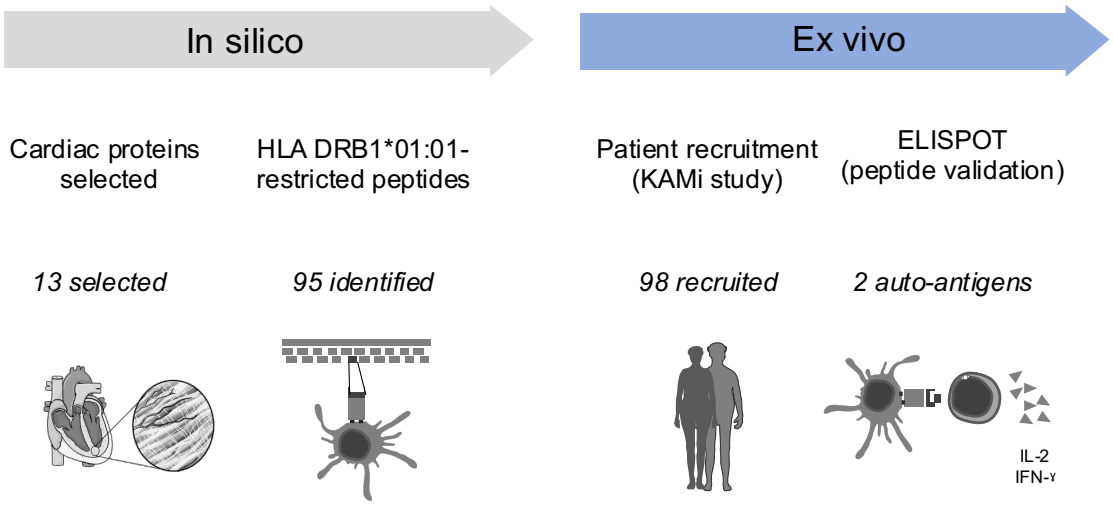

Figure 2 In silico and ex vivo methods

2.2.1 In-Silico-Methods

2.2.1.1 Human Protein Atlas

Protein expression data from the Human Protein Atlas was used to characterize protein expression in the heart. [58,59] We then selected proteins, which show a high expression in the heart relative to other tissues and which we deemed likely to be an important autoantigen in the context of myocardial diseases. These proteins include seven myocardial proteins, two heat-shock-proteins with, which are associated with autoimmune processes, and four proteins of the clotting cascade.

2.2.1.2 UniProt

After selection of peptides, all peptide sequences were gathered from the UniProt-database for further in-silico-analysis. The most recent FASTA sequence for „Homo sapiens (human)“ was used.[60]

2.2.1.3 MHCII-binding prediction

The HLA*DRB1*0101 was chosen as a member of the “main DR”-superfamily for predicting peptide-binding affinity.[61]

MHC II-binding predictions for all selected proteins were generated with the Epitope-prediction tool available on the „Immune Epitope Database“ in October 2017.[62,63] We used the full amino acid sequence for all proteins and generated binding-affinities using the „IEDB-recommend“ prediction methods. Peptides with a percentile-rank smaller than 10.00 were selected for further characterization. All peptides with the same “peptide-core” were grouped together.

2.2.1.4 Protein Cleavage prediction

All selected proteins were also analyzed for protein-cleavage to identify likely processed epitopes. This was done using the “PROSPER: Protease specificity prediction server”[64]. Peptides were excluded from further analysis, when cleavage sites for cathepsin B, D, S or L were present.

2.2.2 Clinical work

2.2.2.1 Study design

To translate our preclinical findings to the clinic and verify our in-silico predictions, the “KAMi”-study (“**K**ardiale **A**ntigene nach **M**yokard**i**nfarkt”) was designed and conducted as part of this work. The aim of this study was, to recruit patients with similar cardiovascular risk-factors either after myocardial infarction or without prior cardiovascular events. Immunological differences observed between the groups are likely caused by the myocardial infarction suffered by patients in the MI-group and not by pre-existing myocardial damage, as this should be comparable between groups. The inclusion and exclusion criteria for the study are shown in Table 1.

MI-Group	ctrl-Group
Inclusion criteria	
Informed Consent	
Age 18-100 years	
Myocardial Infarction with at least one Troponin above 99th percentile	Elective Coronary angiography
	No elevation in heart-enzymes
	At least one cardiac risk factor
Exclusion criteria	
Active tumor	
Hematological disease	
Pathology involving the (skeletal-)muscle	
Immunosuppressive disease or therapy (e.g., steroids)	
Acute or chronic infection	

Table 1 KAMi study design

All patients were recruited in the “MedEins” (Medical clinic 1 of the University Hospital of Würzburg) between 2018 and 2020.

Patients were identified from all patients admitted to the “MedEins” according to the study criteria. Patients were then informed about the study and, if willing to participate, included in the study on the next day. Blood was taken and processed further in the lab. Patients were included on the day after coronary intervention (control group) or 3-8 days after MI (myocardial infarction group).

The study was approved by the Ethics committee with number (304/17-m3) and meets the criteria of the declaration of Helsinki.

2.2.2.2 Blood collection

After patients gave informed consent, a peripheral-venous blood draw was performed by experienced personnel according to usual clinical practice with respect of the hygiene standards.

4x 8ml blood was drawn directly in four collection-tubes ("BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube - Sodium Heparin") and transferred to the laboratory. The blood was stored dark and at room temperature for not more than 2 hours before cell-isolation.

2.2.3 In-vitro methods

2.2.3.1 Peptide handling

Custom peptides were shipped in a lyophilized state from the manufacturer. Upon arrival in the lab, they were stored at -20°C.

To facilitate the in vitro experiments, peptides were dissolved in DMSO and dissolved in purified water to adjust to the appropriate peptide concentration. 96-well plates were pre-planned for the appropriate experiment design and 7,5 µl peptide was plated per well, corresponding to 1/20th of final volume. Prepared plates were stored at -80°C.

2.2.3.2 Cell Isolation

Blood was collected directly in CPT-Tubes, which facilitate isolation of PBMC from full blood.

Tubes were centrifuged at 1,800 g for 20 minutes according to manufacturer's recommendations. Supernatant, which contains plasma, was carefully transferred to cryovials and aliquoted in four separate tubes. The CPT-tube was

then recapped and inverted two times to resuspend periphery blood mononuclear cells (PBMCs). Cells were then decanted into a falcon tube and pooled for each individual patient. After adding pre-warmed, supplemented RPMI-medium, cells were centrifuged at 330g for 10 minutes. The supernatant was decanted, and cells were resuspended and washed in 50ml warm RPMI medium again. After taking a sample for cell-counting, centrifugation at 330g for 10 minutes was performed again. Finally, the supernatant was decanted, and cells were processed in subsequent assays or prepared for cryo-preservation.

2.2.3.3 Cell freezing

The “CTL-Cryo™ ABC Media Kit” (CTL) was used for cryo-preservation of isolated human PBMCs.

“CTL-Cryo™ A” was mixed 0,8:0,2 with “CTL-Cryo™ B” as recommended and warmed in a 37°C CO_2-Incubator. Isolated PBMCs were then resuspended in 3ml pre-warmed “CTL-Cryo™ C”. 3ml “Cryo-AB”-mix was slowly added over a time period of about two minutes and the cell-suspension was aliquoted in six cryovials and kept in a “Mr. Frosty” at -80°C over night. Samples were then transferred to liquid nitrogen for prolonged preservation.

2.2.3.4 Cell thawing

To use PBMCs in subsequent assays, a thawing protocol was established to assure high viability of cells. We followed recommendations published before.[65]

Cryovials were selected from the stock-list and transferred to the cell-culture lab on dry ice. The cryovials were then warmed in a 37°C-water-bath for 8 minutes. Further work was done under a laminar flow hood. Tubes were inverted twice to resuspend cells and the content was transferred to a 50ml tube. All cryovials from the same patient were pooled. The cryovial was then washed with 1ml warm RPMI-medium and the medium was also added to the transferred

cells to increase cell yield. Another 8ml medium was added for each cryovial thawed. Of this, the first 3ml were added slowly (1ml every 5 seconds while gently whirling the tube). The cells were then washed twice by centrifugation at 330g for 5 minutes and thereafter used in further experiments.

2.2.3.5 ELISPOT-Assay

To quantify the immune response after stimulation with candidate autoantigens, we performed ELISPOT-assays with the isolated PBMCs using the "IL-2/IFN-γ"-Kit (CTL).

Provided 96-well-plates were pre-coated with capture antibodies. For this, 15 µl 70% Ethanol was added to each well. Immediately, 150µl PBS was added and the plate decanted. Plates were washed again with 150µl PBS. After this activation step, 80µl Capture Solution, which was prepared according to manufacturer's recommendations, was added to all wells. The plate was sealed and kept at 4°C over night.

After incubation, the seal was removed, and plates washed once again with 150µl PBS. Prepared peptides were diluted in 75µl "CTL-Test-Medium" (CTL) and added to the ELISPOT-plates at a concentration of 2µg/ml. The plates were transferred to a 37°C/5% CO_2-incubator while cells were processed.

PBMCs of selected patients were thawed (→ Cell thawing) and adjusted to a concentration of $4x10^6$ / ml. 75µl of cells were added to each well of the ELISPOT-plate and plates were incubated at 37°C/5% CO_2 for 24 hours with a final peptide concentration of 1µg / ml and a cell count of 300.000 cells / well.

After incubation, 50µl / well of supernatant was transferred to a fresh 96-well-plate and sealed. The supernatant was then kept at -20°C for later experiments. The ELISPOT-plate was decanted and washed with PBS.

Subsequent staining and washing were performed according to the provided protocol.

Plates were then air-dried over night before analysis with a CPT-Elispot-Reader.

2.2.3.6 Legendplex-Assay

As we were only able to test for two secreted cytokines at a time with our ELISPOT-kits, it is quite likely that we miss a cytokine response of a different type. To avoid this, we tested the ELISPOT-supernatant of two patients in a "LEGENDplex-Assay".

Experiments were performed according to the manufacturer's recommendations using the LEGENDplex™ Human Th Panel (13-plex), (BioLegend).

2.2.3.7 ELISA

As the cytokine IL-6 was not included in the Elispot-Kit, we measured IL-6 levels by ELISA in the supernatant after Elispot-Assay.

"ELISA MAX™ Standard Set Human IL-6" (BioLegend) were purchased and the experiments were performed according to manufacturer's recommendations. Supernatant from the ELISPOT-Assays was used at a 15-fold dilution.

2.2.3.8 DNA-Isolation

DNA isolation was performed from isolated PBMCs using the "GeneJET Genomic DNA Purification Kit" (Thermo Scientific) according to the manufacturer's recommendations for Mammalian Blood samples.

2.2.3.9 HLA-genotyping

DNA was isolated in the lab and samples were provided to the "Institute for Transfusion Medicine and Haemotherapy" of the University Hospital of Würzburg for HLA genotyping.

2.2.4 Data-Analysis

2.2.4.1 ELISPOT-Analysis

To quantify cytokine responses after Elispot-Assays, the „ImmunoSpot® S6 CORE"-Elispot-Reader (CTL) was used. Spots were counted using the "Dual Color" software using the automatic spot detection algorithm.

Data was then transferred to Microsoft Excel and medium-control wells were used to correct for cytokine background signal.

2.2.4.2 Peptide Score

For experiments, where peptide matrices were designed, we calculated a peptide score for each single peptide, to estimate which peptides are most likely to cause the observed results. Pearson's r was calculated for correlation of pools, containing a specific peptide. The resulting factor was then multiplied by the cytokine response in every of the two pools containing the peptide. This calculation was repeated for every single peptide and peptides with the highest score were deemed most likely to cause the observed cytokine response.

2.2.4.3 Statistics

Unless otherwise stated, the results are displayed as the mean ± the standard error of the mean (SEM) alongside with the distribution of all individual values in each group. The sample size for each group is described in the legend of each graph. The statistical data analyses were performed with GraphPad Prism. Unless otherwise stated, the following tests were used in this work: For comparisons between two groups of data following a normal distribution, we used an unpaired two-tailed t-test. For multiple comparisons between more than

two groups, one- or two-way analyses of variance (ANOVA) were conducted followed by a post-hoc test which is always specified in the legend of each graph. Differences were considered significant when $p < 0.05$ (*).

For Elispot results from clinical cohorts, an upper baseline was established by calculating the 95% confidence interval of IFN-γ-secretion of the respective control group. Patients were then classified as “responders” and “non-responders” based on this threshold. A fisher’s exact test was then performed to compare the frequency of responses and the two-tailed-p-value is reported.

3 Results

3.1 Antigen Atlas – Peptide predictions

As there is a vast number of potential autoantigens in the cardiac context, we set out to narrow down the possible candidates before performing experimental studies.

To facilitate epitope discovery, we designed an in-silico analysis pipeline as described above and narrowed down the epitopes we deemed most likely to function as autoantigens to 95 peptides spread across 13 proteins (Table 2). An example of the resulting peptide library is given for the protein ADRB1 (Table 3). The full antigen atlas is provided as an attachment to this work (Supplementary Material 1).

Heart-specific Antigens		
Protein	**Full name**	**# predicted epitopes**
ACTC1	Actin alpha 1, cardiac isoform	4
ADRB1	Adrenoceptor Beta 1	22
MYBPC3	Myosin binding protein C, cardiac	16
MYH6	Myosin Heavy Chain 6, cardiac isoform	24
MHL2	Myosin light Chain 2 (Ventricular)	1
TNNI3	Troponin I type3, cardiac isoform	3
TNNT2	Troponin T type2, cardiac isoform	1
Inflammation-associated-Antigens		
HSPB3	Heat-Shock Protein 27	4
HSPA1A	Heat-Shock Protein 72	3
Clot-derived antigens		
FGA	Fibrinogen-alpha chain	7
FGB	Fibrinogen-beta chain	4
FGG	Fibrinogen-gamma chain	4
Modified proteins		
CIT	Citrullinated Fibrinogen	2

Table 2 Cardiac Antigen Atlas Overview

Peptide position	Peptide Sequence	Percentile
ADRB1$_{28-44}$	TA**ARLLVPASP**PASLLP	5.46
ADRB1$_{63-77}$	**LLMALIVLL**IVAGNV	9.01
ADRB1$_{67-81}$	LIV**LLIVAGNVL**VIV	1.36
ADRB1$_{75-89}$	**GNVLVIVAI**AKTPRL	8.28
ADRB1$_{76-90}$	NV**LVIVAIAKT**PRLQ	9.87
ADRB1$_{92-106}$	**LTNLFIMSL**ASADLV	0.88
ADRB1$_{93-107}$	TN**LFIMSLASA**DLVM	0.77
ADRB1$_{96-110}$	F**IMSLASADL**VMGLL	3.24
ADRB1$_{97-111}$	IMSLAS**ADLVMGLLV**	5.59
ADRB1$_{107-121}$	MGL**LVVPFGATI**VVW	7.51
ADRB1$_{153-167}$	ALD**RYLAITSPF**RYQ	6.65
ADRB1$_{164-178}$	FR**YQSLLTRAR**ARGL	4.95
ADRB1$_{167-181}$	**QSLLTRARA**RGLVCT	7.48
ADRB1$_{168-182}$	SL**LTRARARGL**VCTV	8.87
ADRB1$_{177-191}$	GLV**CTVWAISAL**VSF	8.67
ADRB1$_{215-229}$	CCD**FVTNRAYAI**ASS	0.62
ADRB1$_{220-234}$	**TNRAYAIAS**SVVSFY	3.24
ADRB1$_{221-235}$	NRAYAI**ASSVVSFYV**	3.02
ADRB1$_{311-325}$	**PSRLVALRE**QKALKT	6.00
ADRB1$_{312-326}$	SRL**VALREQKAL**KTL	5.59
ADRB1$_{334-348}$	TLCWLP**FFLANVVKA**	8.67
ADRB1$_{335-349}$	LCWLPF**FLANVVKAF**	6.52

Table 3 Predicted epitopes for the protein ADRB1
The predictions for DRB1*01*01 are given with the location in the protein, amino acid sequence and affinity percentile. Bold text indicates the “peptide core”.

Epitope prediction resulted in 22 likely immunogenic candidates for ADRB1 with different binding affinity percentiles of up to 10 percent. All epitopes were of 15 amino acids in length and spanned the whole protein with some clustering and overlap.

3.2 Clinical cohort

To investigate the role of T cells after MI, 98 patients were included in the KAMi-study. Inclusion criteria and study design have been described above. General patient characteristics as well as biochemical markers related to myocardial infarction were collected.

Patients in the MI cohort presented with STEMI in 73% of the cases and had a mean age of 62.49 years. Control patients had a mean age of 63.47 years.

As this is an exploratory study, a subset of patients was used for the different experiments and results cannot be generalized for the whole study population. Patients were selected arbitrarily based on inclusion time to be included in each one of the experiments and the number of included patients is reported for every experiment.

3.3 Elispot Analysis

To verify the relevance of predicted peptides, a dual-color Elispot assay was performed for the cytokines IL-2 and IFN-γ as described above. All predicted epitopes for each protein were pooled and isolated PBMCs from patients were stimulated with the respective peptide pool. IL-2 and IFN-γ were measured and the summarized, baseline-corrected results are shown (Figure 3 + Figure 4). IL-2 cytokine responses are comparable between groups for all tested peptides and controls and no biologically meaningful difference could be shown.

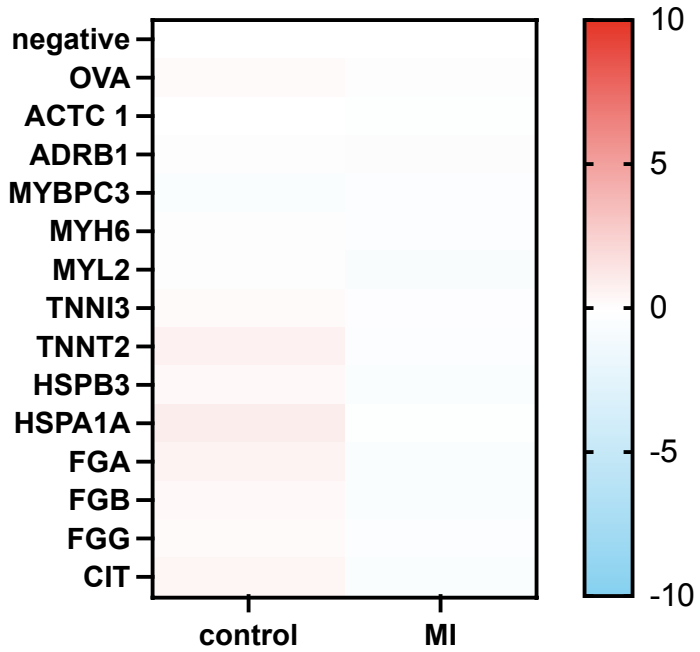

Figure 3 IL-2 secretion after pooled peptide stimulation
PBMCs were stimulated with pooled epitopes for each protein. Mean number of cytokine secreting cells is reported for each pool. Results are baseline corrected.
patients included in this analysis: control n = 12, MI n=20

There was no significant difference in the IFN-γ response between groups for the negative control (OVA-peptide) (mean response 0.06 for control vs. 0.67 for MI).

No significant difference between groups was shown for the tested peptide-pools across all patients. However, there was a subset of patients responding to peptides in the ADRB1-pool, causing a difference in the pooled response (Figure 4). Mean number of IFN-γ secreting cells was 5.48 in the MI group compared to 0.97 in the control group.

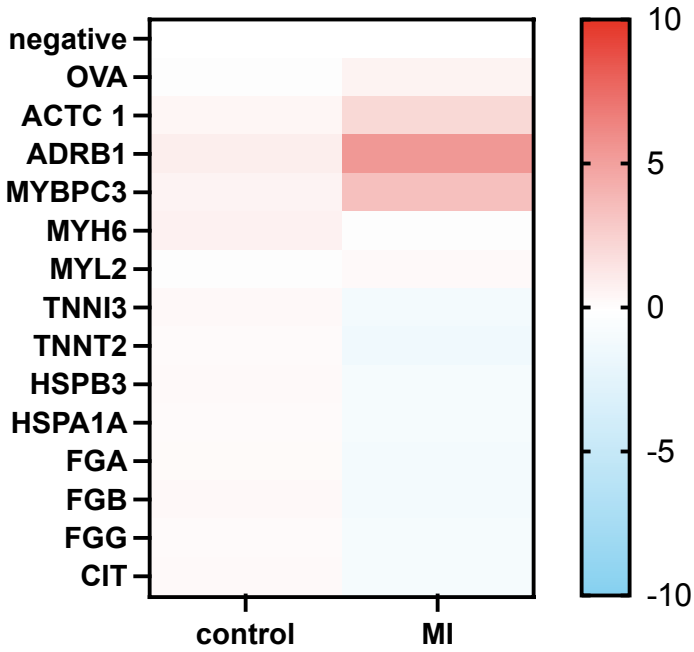

Figure 4 IFN-γ secretion after pooled peptide stimulation
PBMCs were stimulated with pooled epitopes for each protein. Mean number of cytokine secreting cells is reported for each pool. Results are baseline corrected.
patients included in this analysis: control n = 12, MI n=20

3.4 Legendplex-Assay

As only IL-2 and IFN-γ responses were measured in the Elispot assay, we deemed it possible to miss cytokine responses of a different phenotype. As we are interested in any autoreactivity against cardiac antigens, we set out to uncover possible oversights. Thus, a Legendplex-Assay was performed to measure an extensive panel of cytokines and explore, whether another T cell phenotype than Th1 was present after autoantigen stimulation (Figure 5).

The only cytokine secretion we were able to detect in peptide-stimulated wells was that of IL-6, which was most pronounced after stimulation with MYBPC3. Slightly lower but still elevated IL-6 levels were observed in response to stimulation with ADRB1 and MYH6. No secretion of other cytokines that was reasonably above baseline was detected.

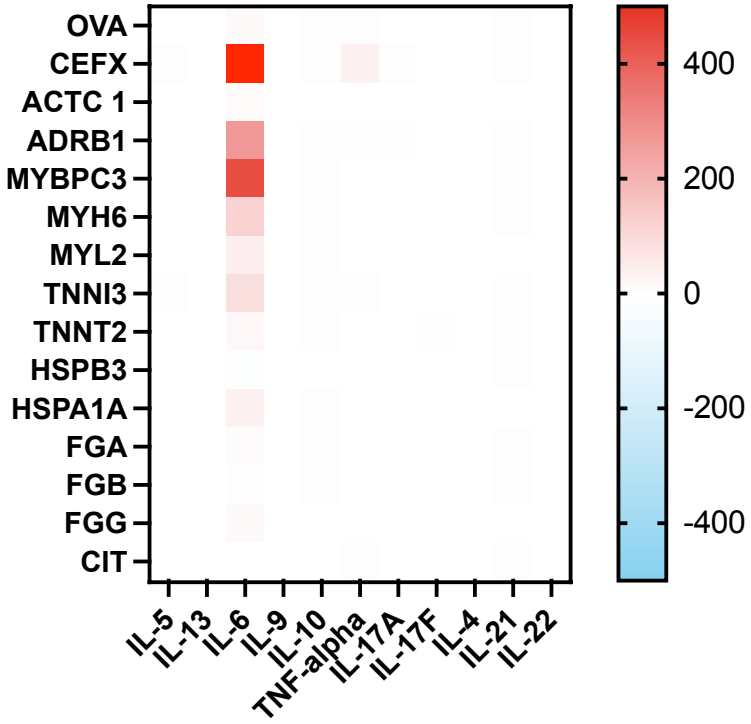

Figure 5 Legendplex assay after peptide pool stimulation
Baseline-corrected values, pg/mL
MI high responders, n=2

3.5 IL-6 Elisa

The Legendplex assay turned up evidence, that an IL-6 response might be a strong readout after antigenic stimulation. To verify this, we measured IL-6 levels after antigen stimulation from supernatants collected after previous Elispot experiments as described.

IL-6 levels were comparable in the negative control OVA peptide for both groups (-154.2 for control vs. 107.8 for MI, pg/ml). Similar to the previously shown data, there was a subset of patients in the MI group, who responded to pooled peptides of ADRB1, causing a rise in mean response (659.5 for control vs. 2907.9 for MI, pg/ml). Another curious finding was a higher response after stimulation with MYH6 peptides (729.3 for control vs. 3115.4 for MI, pg/ml), a finding that is in line with findings from the mouse model.[54] A lower, but still elevated response was shown after stimulation with MYPBC3 (460.4 for control vs. 1780 for MI, pg/ml).

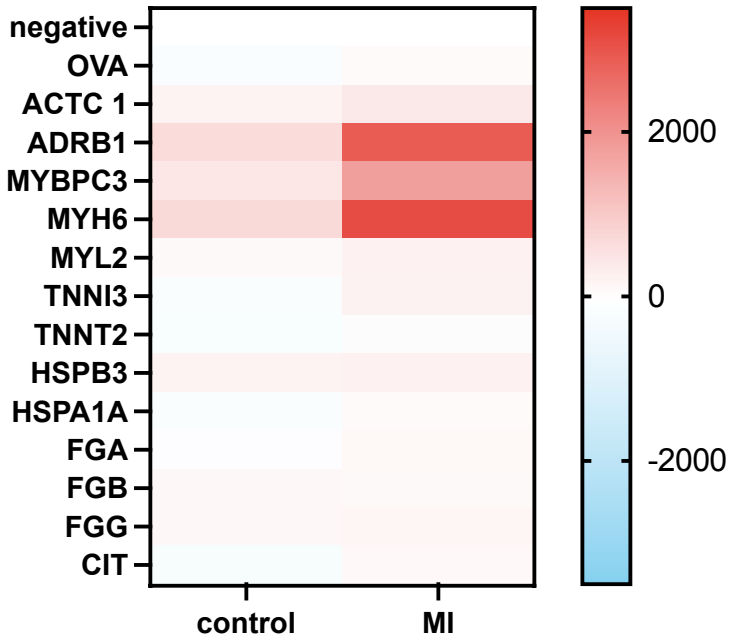

Figure 6 IL-6 secretion after peptide pool stimulation
Isolated PBMCs were stimulated with pooled peptides for each protein and IL-6 levels were measured in the supernatant. Mean values are plotted in pg/ml. Values are baseline corrected.
Patients included in this analysis: control n = 6, MI n=9

3.6 Single protein responses

As we observed some moderate differences in mean cytokine responses between the groups, we started to investigate, whether these differences were caused by a random variation or by a meaningful immune response that only occurs in a subset of patients and is thus dampened by pooling of the results.

The highest difference between groups was shown for ADRB1 (Figure 7). There is a substantial subset of patients in both groups, that do not respond to peptide stimulation. However, some patients in the MI group show a cytokine secretion after stimulation with ADRB1 derived peptides, suggesting a meaningful immune response, that does not occur in the control group. To investigate the difference between patient, all patients were grouped into “responders” and “non-responders” as described (Statistics).

With a calculated threshold of 1.69 IFN-γ-secreting cells/well, 9/20 patients in the MI group are classified as responders, whereas only 2/12 patients in the control group responded to stimulation (45% vs. 17%, p=0.139).

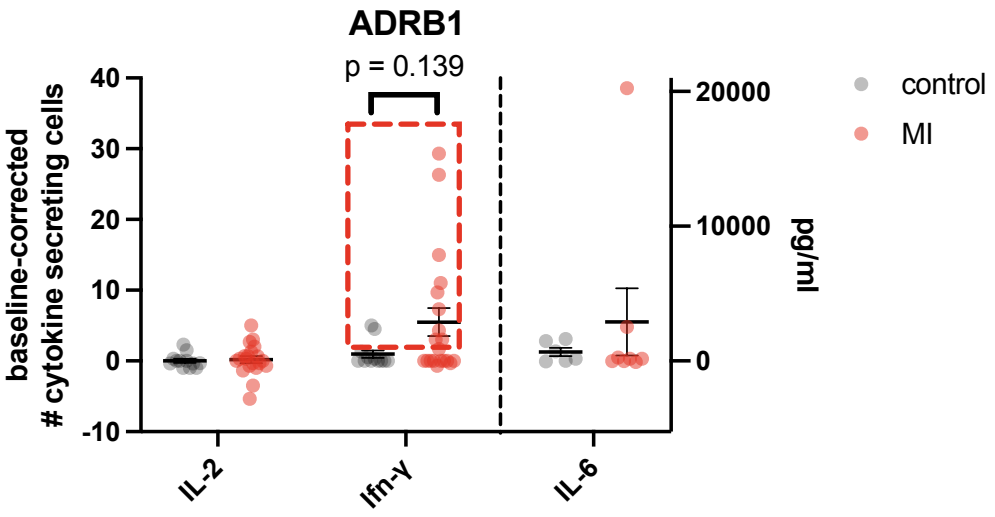

Figure 7 ADRB1 peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

A comparison of responders vs. non-responders for all peptide pools shows few remarkable differences in responses. The strongest difference in MI vs. control is observed in response to ADRB1. (Table 4)

	NK	N = 12	MI	N = 20
	# of responders		# of responders	
OVA	2	17%	4	20%
ACTC 1	3	25%	4	20%
ADRB1	2	17%	9	45%
MYBPC3	2	17%	6	30%
MYH6	1	8%	2	10%
MYL2	1	8%	4	20%
TNNI3	3	25%	0	0%
TNNT2	3	25%	1	5%
HSPB3	1	8%	2	10%
HSPA1A	2	17%	2	10%
FGA	2	17%	2	10%
FGB	3	25%	1	5%
FGG	2	17%	3	15%
CIT	2	17%	2	10%

Table 4 Responding patients after antigen stimulation
Responding patients were calculated as described above for every peptide pool. Number of responding patients and percentage of responders vs. non-responders is reported.

The highest difference of responders vs. non-responders is observed after stimulation with ADRB1. A higher fraction of responding patients in the MI group is also observed for a few other proteins. The data for these is also shown for comparison, highlighting that the ADRB1 response is both strongest and most common (Figure 8 - Figure 12).

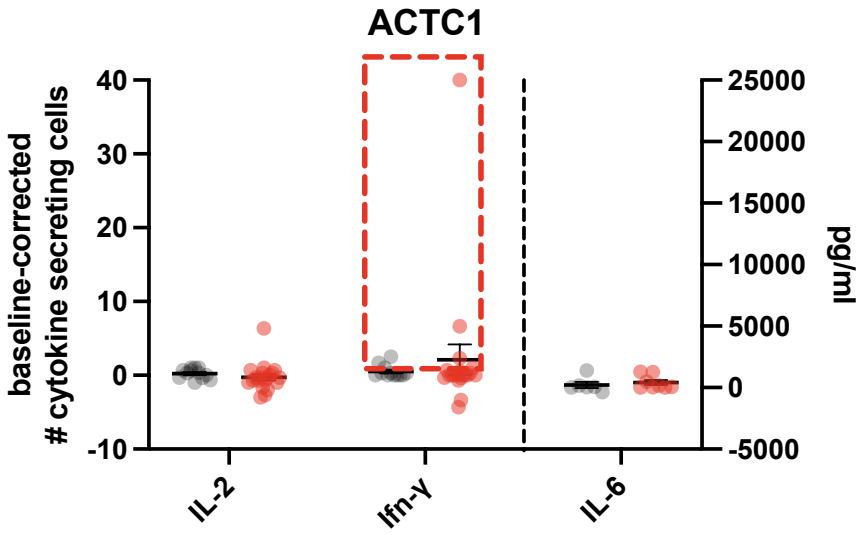

Figure 8 ACTC1 peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

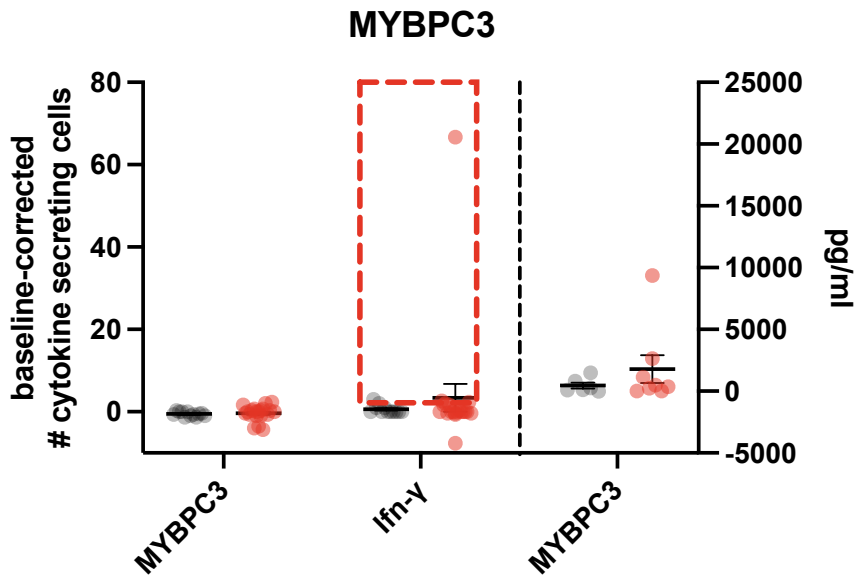

Figure 9 MYBPC3 peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

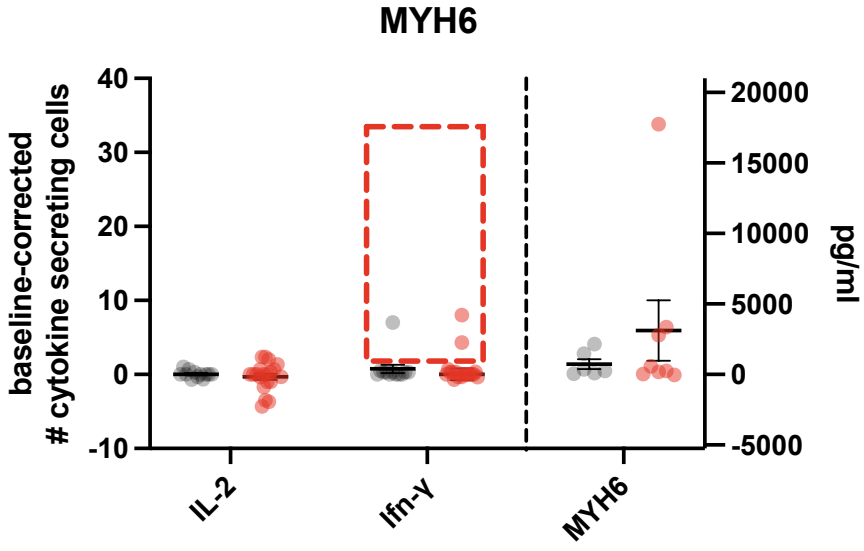

Figure 10 MYH6 peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

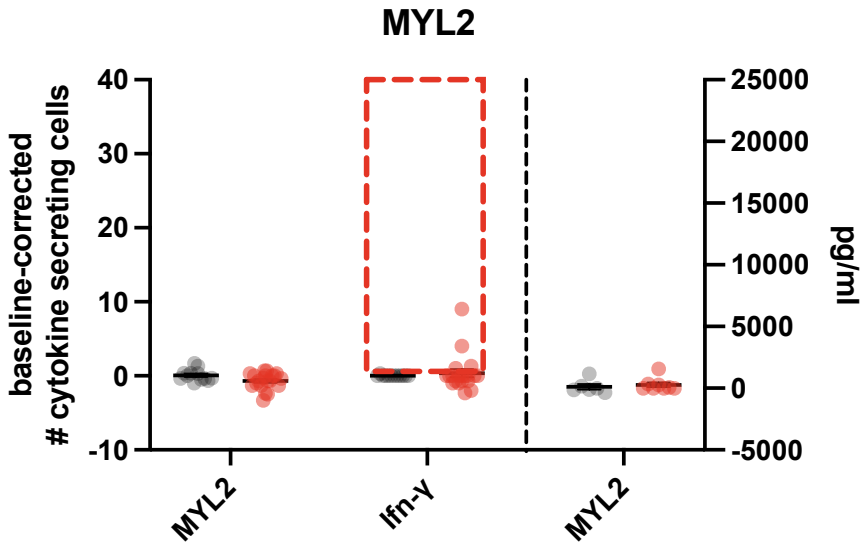

Figure 11 MYL2 peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

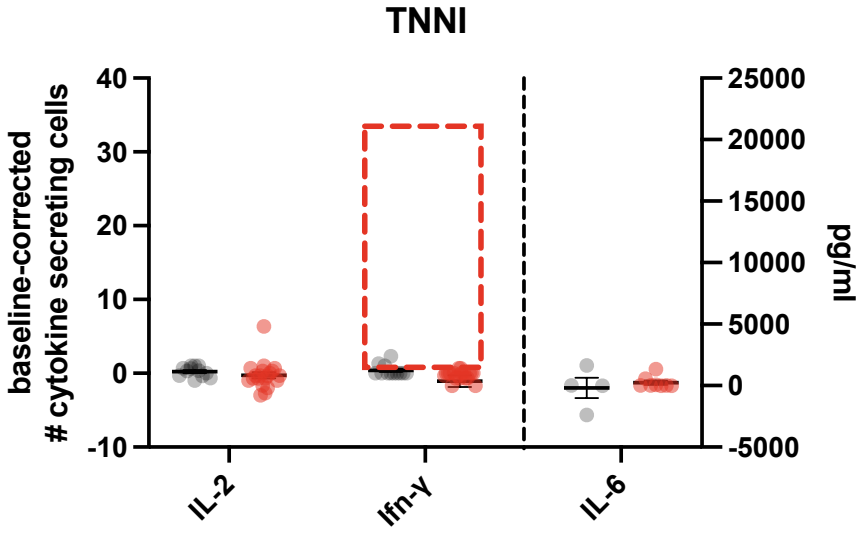

Figure 12 TNNI peptide pool
PBMCs were stimulated with 22 pooled peptides for ADRB1. Mean number of cytokine secreting cells is reported for IL-2 and IFN-γ. Cytokine concentration in pg/mL in the supernatant is reported for IL-6. Results are baseline corrected.
Patients included in this analysis: control n = 12, MI n=20

3.7 Single Epitope discovery

3.7.1 Matrix experiments

As there is only a subset of responders and the immunogenicity of tested peptides seems to differ between patients, we wondered, whether all responders shared a common immunodominant epitope or whether a heterogenous response prevailed.

To further dissect the antigenicity of ADRB1 in the context of myocardial infarction, we designed an array of peptide pools, covering different subsets of ADRB1-epitopes. As we have only limited biomaterial available, it would not be feasible to test all predicted epitopes separately. Thus, we designed peptide pools, which limit the number of necessary tests, but allow the identification of single peptides causing cytokine responses we might observe. These pools were used to stimulate isolated PBMCs as described previously and a peptide score for each single epitope was calculated.

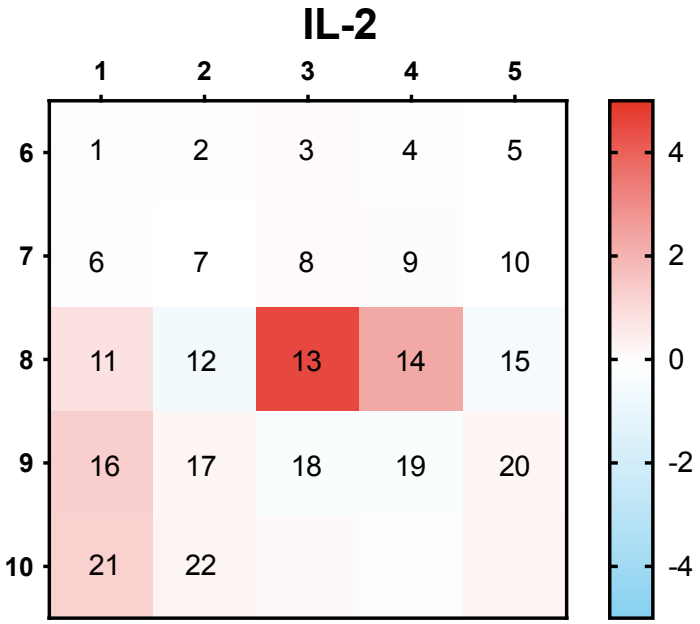

Figure 13 ADRB1 peptide pool matrix – IL-2
Peptides were grouped into pools 1-10 (rows and columns) including all peptides in the respective row/column. PBMCs were stimulated with pools, cytokine secretion measured by Elispot and results were used to calculate a peptide score for each single peptide. A heatmap of the calculated peptide-scores is reported, with a higher peptide score corresponding to a higher likelihood of immunodominance.
Patients used: MI responders, n = 6

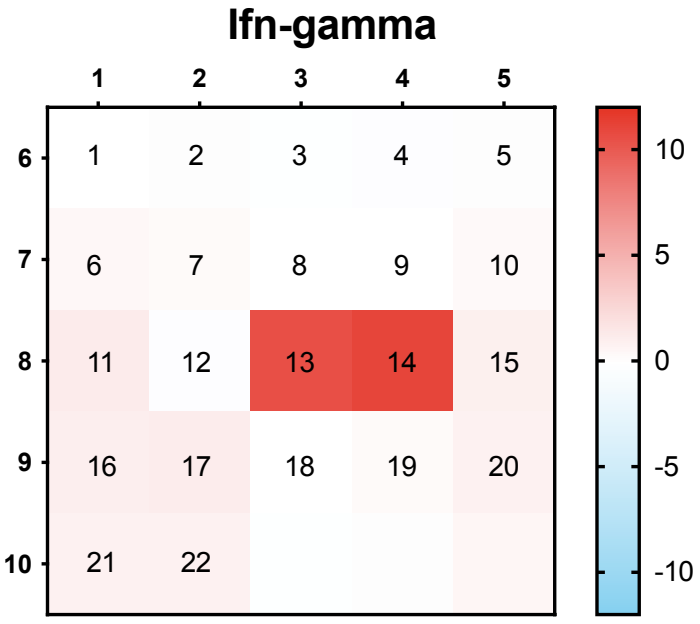

Figure 14 ADRB1 peptide pool matrix – IFN-γ
Peptides were grouped into pools 1-10 (rows and columns) including all peptides in the respective row/column. PBMCs were stimulated with pools, cytokine secretion measured by Elispot, and results were used to calculate a peptide score for each single peptide. A heatmap of the calculated peptide-scores is reported, with a higher peptide score corresponding to a higher likelihood of immunodominance.
Patients used: MI responders, n = 6

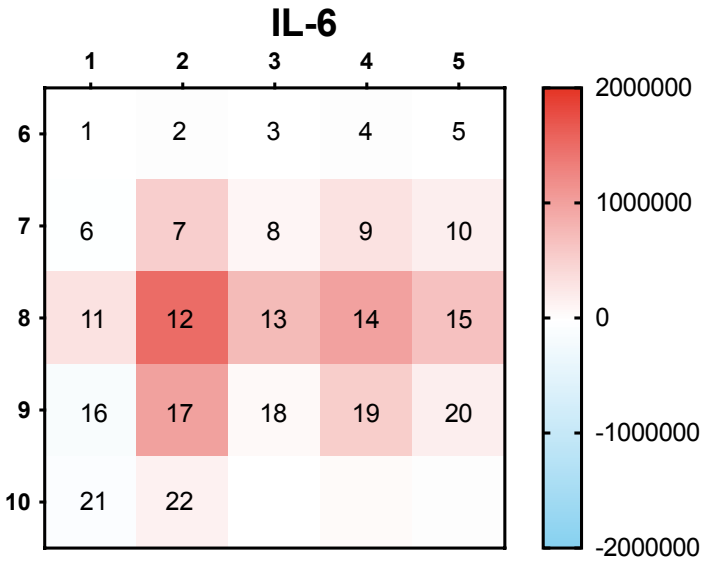

Figure 15 ADRB1 peptide pool matrix – IL-6
Peptides were grouped into pools 1-10 (rows and columns) including all peptides in the respective row/column. PBMCs were stimulated with pools, cytokine secretion measured by Elisa and results were used to calculate a peptide score for each single peptide. A heatmap of the calculated peptide-scores is reported, with a higher peptide score corresponding to a higher likelihood of immunodominance.
Patients used: MI responders, n = 6

Experimental evidence showed no single immunodominant epitope, but two peptides, which caused comparable T cell activation. Interestingly, these peptides show a strong overlap and share most of their amino acid sequence (Figure 16).

Figure 16 ADRB1 epitopes sequence
Predicted peptides 11-15 are shown and mapped to the corresponding amino acid sequence. Peptides cover the ADRB1 sequence from position 153 to 191

3.7.2 Single peptide validation

The identified peptides were remanufactured at a high purity and used to confirm the relevance of the predicted epitopes.

The results were consistent with predicted antigenicity, but there was still only a response in a subset of patients (Figure 17 + Figure 18). The response was most pronounced against $ADRB1_{167\text{-}181}$, where there were more responding patients in the MI cohort compared to the control cohort (6/31 vs. 1/39, p=0.039).

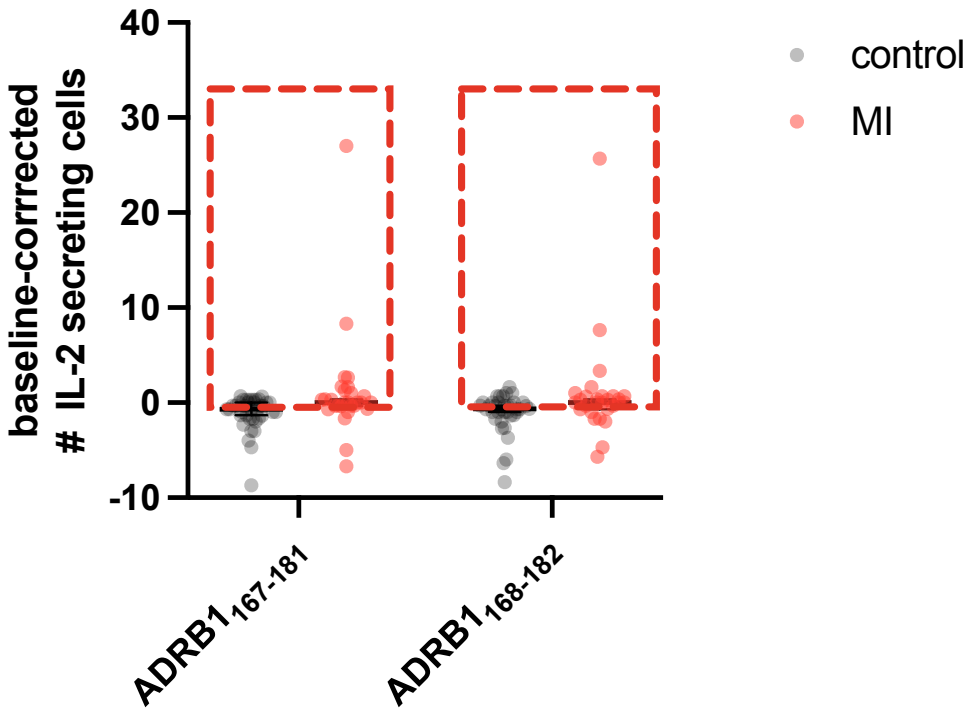

Figure 17 ADRB1 single peptide stimulation – IL-2
PBMCs were stimulated with 3 single peptides from ADRB1. Mean number of cytokine secreting cells from triplicates is reported. Results are baseline corrected.
Patients included in this analysis: control n = 38, MI n=31

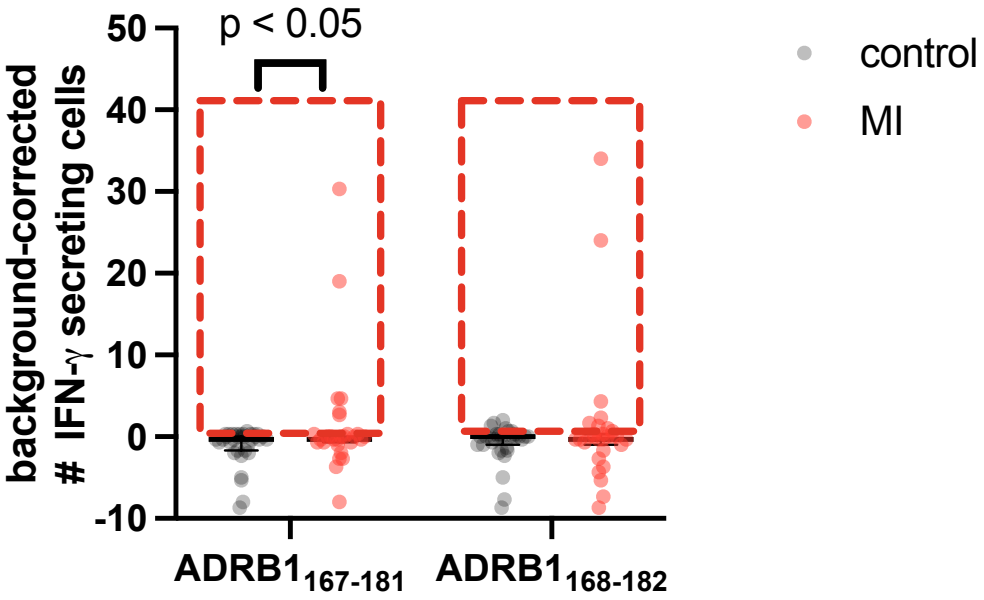

Figure 18 ADRB1 single peptide stimulation – IFN-γ
PBMCs were stimulated with 3 single peptides from ADRB1. Mean number of cytokine secreting cells from triplicates is reported. Results are baseline corrected.
Patients included in this analysis: control n = 38, MI n=31

3.8 HLA-sequencing

As we observed a response towards the autoantigenic peptides in only a subset of MI patients, we started to investigate, which factors might separate the groups of “responders” and “non-responders”. As HLA-molecules are important for presentation of antigens, it seemed likely that HLA-heterogeneity might influence presentation and thus response towards the predicted autoantigens. We decided to perform sequencing of the HLA-DRB1 gene locus, as this is the most common beta-subunit in the HLA-DR group and is known to influence autoimmune reactions.

HLA-sequencing of the HLA-DR-locus revealed an interesting division of patients into HLA-groups.

When analyzing the previously described Elispot-results, there is a clear influence of HLA on reactivity against ADRB1. HLA-DRB1*13-carriers show a response in 5/8 cases in the MI group and in 0/3 cases in the control group, after stimulation with pooled ADRB1 peptides (Figure 19).

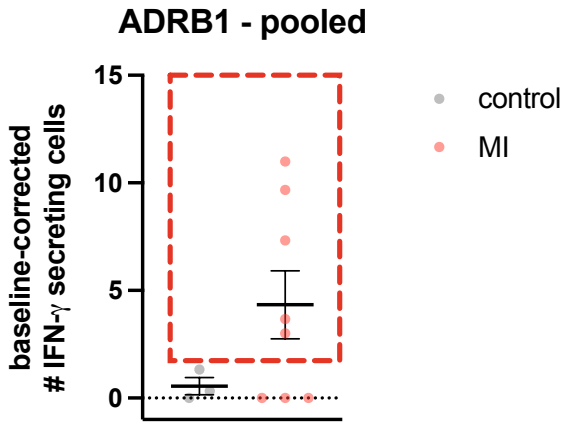

Figure 19 ADRB1 peptide pool – HLA-DRB1*13 carriers
PBMCs were stimulated with 22 pooled peptides for ADRB1. Experiments were done in triplicates and mean number of cytokine secreting cells is reported. Results are baseline corrected.
Patients included in this analysis: control n = 3, MI n=8

After stimulation with single peptides, most of the responding patients are carriers of HLA-DRB1*13 as well.

An IFN-γ secretion is observed in 6/9 HLA-DRB1*13-carriers in the MI group, after stimulation with the most immunogenic ADRB1-epitope (167-181). MI patients, who were negative for HLA-DRB1*13 showed no response out of 22 patients. (Figure 20). As expected from previous experiments, IL-2 response shows a similar trend, but overall lower secretion (Figure 21).

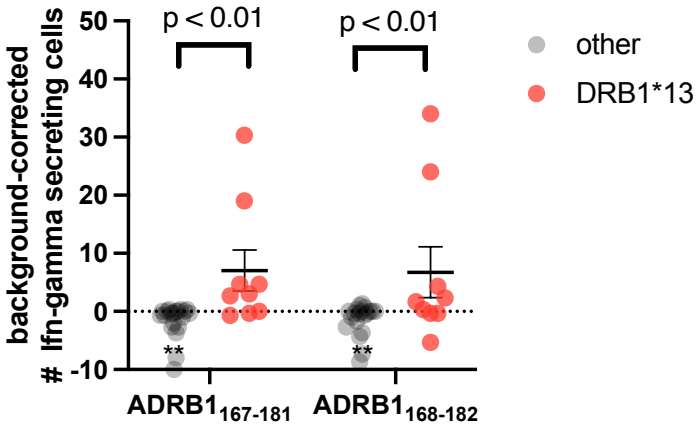

Figure 20 ADRB1 single peptide stimulation – IFN-γ
PBMCs were stimulated with 3 single peptides from ADRB1. Mean number of cytokine secreting cells from triplicates is reported. Results are baseline corrected. Stars indicate results outside axis limit.
Patients included in this analysis: MI other alleles n = 22, MI carrier of allele DRB1*13 n=9

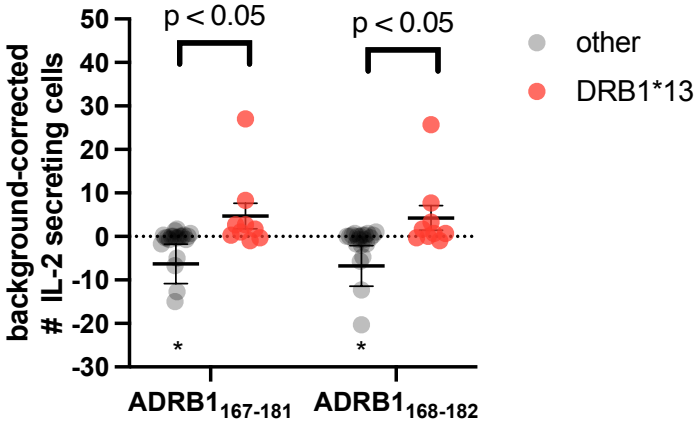

Figure 21 ADRB1 single peptide stimulation – IL-2
PBMCs were stimulated with 3 single peptides from ADRB1. Mean number of cytokine secreting cells from triplicates is reported. Results are baseline corrected. Stars indicate results outside axis limit. Patients included in this analysis: MI other alleles n = 22, MI carrier of allele DRB1*13 n=9

Interestingly, patients in the MI group were almost twice as likely to be HLA-DRB1*13 carriers than patients in the control group. There were 9 out of 49 patients in the control group who were carriers of at least one allele of HLA-DRB1*13, but 15 out of 47 in the MI group of this genotype. This difference was not statistically significant, but was also not observed for other allele groups (HLA-DRB1*01 and HLA-DRB1*04 as an example, Figure 23 + Figure 24 HLA-DRB1*04 carrier distribution.).

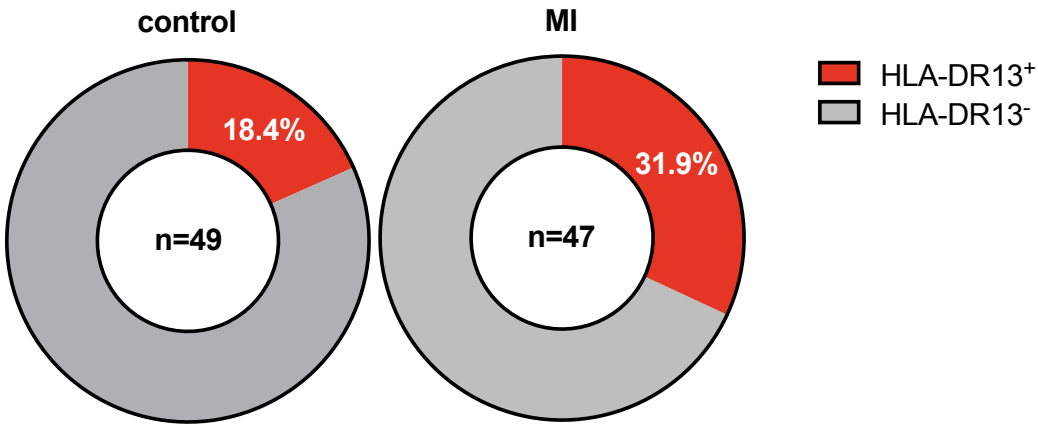

Figure 22 HLA-DRB1*13 carrier distribution

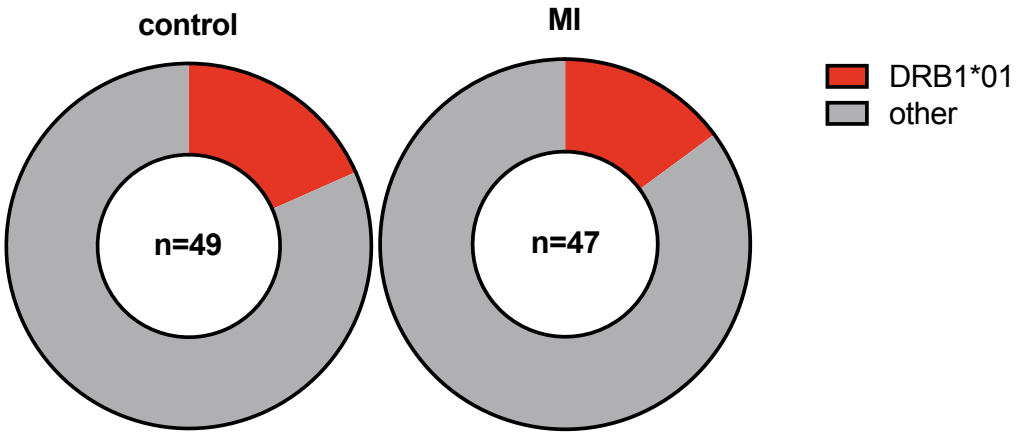

Figure 23 HLA-DRB1*01 carrier distribution.

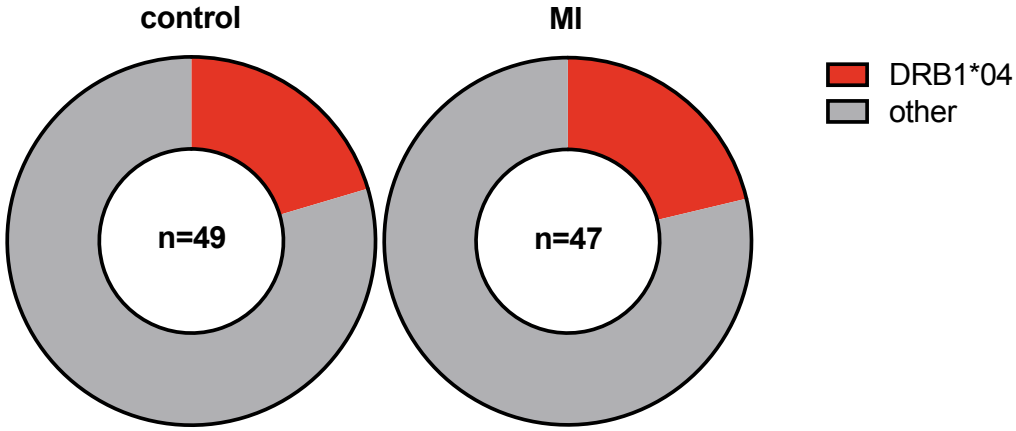

Figure 24 HLA-DRB1*04 carrier distribution.

4 Discussion

To unravel the complicated immune response after myocardial infarction and contribute to the basic understanding of the healing process, we designed and conducted this study. While our results stand on their own, I want to put them in perspective of current research in immunocardiology to give some outlook on future possibilities we are opening with our discoveries.

Some number crunching interestingly illustrates the great difficulties we faced when looking for human cardiac autoantigens.

Out of all human proteins, 387 show an elevated expression in the heart, making them possible tissue-specific auto-antigens.[59] Any 15 amino acid long peptide out of these protein sequences could function as an antigen, but to be relevant as an autoantigen for T helper cells, an epitope would have to be able to bind to a HLA class II molecule, of which there are over 8500 known variants.[66]

If binding is possible, the resulting pMHC complex can be recognized by a specific TCR.

However, as TCRs are generated randomly, there are over 10^{15} possible receptors that could be of relevance.[67] Out of these possible receptors, every single one could potentially recognize more than one million different peptides, making the amount of interactions almost uncountable.[11]

We were able to identify two epitopes derived from one single cardiac autoantigen, which likely bind to HLA-DRB1*13:01 or HLA-DRB1*13:02 and induce an immune reaction in patients after myocardial infarction. This important discovery lays the groundwork for further characterization of autoreactive T cells after MI and the identification of relevant TCRs.

4.1 ADRB1 autoreactivity in patients after myocardial infarction

In vitro stimulation of PBMCs with a mix of ADRB1 derived peptides caused a measurable IFN-γ secretion from T cells indicating a pro-inflammatory response. This response was not observed in control patients, suggesting a correlation with the ischemic injury of the myocardium.

As we used peripheral blood cells in our study, there are a few caveats one must keep in mind, when interpreting the results.

First, T cells are very numerous and antigen specific cells against any single one antigen are sparse, making the identification of these cells difficult. We show only few cytokine secreting cells in the 10s out of the 100,000s of cells, which were plated in every well. This sparse response is caused by the small number of reactive T cells against a single antigen as well as the limited detection of the cytokine profile. We would only be able to detect reactivity of a specific phenotype and are not able to survey all reactive T cells.

As we assumed a pro-inflammatory response after myocardial infarction to be prevalent, we mainly measured pro-inflammatory cytokines. Indeed, it was possible to show that responding cells secrete the pro-inflammatory cytokines IFNγ and IL-6, but our study was not designed to characterize the phenotype they would have in vivo. Although it looks like we identified a pro-inflammatory subset of T cells, this might be influenced by our experimental setup.

T cell autoantigens are comparably hard to measure, so there is little evidence on their relevance in heart disease. What is easier to measure and thus better studied, is the development of antibodies against self, as these can bind antigen independent of presenting MHC molecules.

Interestingly, there is some evidence for the relevance of anti-ADRB1 in the development of dilative cardiomyopathy in the animal model.[68] Similar antibodies were also shown to be present and predictive in patients with dilative cardiomyopathy as well as some patients with ischemic cardiomyopathy.[69]

As B Cells largely depend on T cell help for differentiation and antibody production, it is reasonable to assume a connection between the observed T cell autoreactivity against ADRB1 and the development of autoantibodies. Further research is needed to uncover possible causalities and investigate, whether a T cell – B Cell axis is of diagnostic or therapeutic relevance in heart disease. With this work, we provide the first evidence for a triggered T cell autoimmunity directed against ADRB1.

4.2 Heterogeneity of immune responses in patients

However, this response was only observed in a subset of patients. At first, this seems to undermine the conclusion that this observation might frequently be triggered by myocardial infarction and that it would thus be a biological meaningful observation. If we keep in mind, that human response to injury and disease is extremely diverse, our findings become very interesting. To show an effect that is present in many, but not all patients, has even bigger translational potential, as it is not only possible to uncover underlying principles, but stratification and personalized treatment of patients become possible.

The vast majority of all available knowledge about the immune system was gathered in animal experiments. To study autoantigens and T cell responses in the mouse model is an elegant endeavor, as most of the heterogeneity and variations that are central to being human can be abstracted away in genetically identical mice. This neat and clean animal model is very far from the heterogenous reality of humans. If we keep this in mind, it is not surprising, that autoantigens, which were shown to be relevant in the mouse model, have not been of importance in our study.[54]

In addition, there is a remarkable importance of the microbiome in our guts for many diseases. Immune responses and tolerance in particular are likely to be impacted by commensal bacteria. As mice are mostly housed in germ-free

facilities and do not commonly have variations in their diet, they are a far more homogenous than our patients.

Both the microbiome as well as the HLA have been shown to be important determinants of autoimmunity. Autoimmunity against MYH6 was shown to be mediated by commensal bacteria in dependence on HLA-genotype in mice and patients.[55]

While it is not trivial to dissect the heterogeneity of human beings and uncover biological principles, we were able to identify a certain HLA allele, DRB1*13, which is necessary for autoreactivity against ADRB1 in our study. In addition, we observed carriers of DRB1*13 to be at a higher risk for MI. While this finding is interesting on its own and might be explained by the autoreactivity we discovered, it is important to keep in mind, that our small study is not suited to make conclusions about genetic risk factors. To investigate this further, larger population studies could be used.

While HLA-DRB1*13 has not been previously suggested to increase the risk of MI, there is some work on the general risk of MI related to other HLA-haplotypes.[70] MI is most often caused by atherosclerosis and as atherosclerosis is thought to be an autoimmune process, it is likely to assume, that HLA plays a big role in its development. We were able to show first hints for HLA-DRB1*13-alleles being a risk factor for development of myocardial infarction.

In contrast, certain DRB1*13-alleles were shown to protect against rheumatoid arthritis.[71]

As scientific papers mostly focus on one specific subject or disease, it often seems like we classify patients into “good and healthy” and “bad and genetically worse off”. However, it is important to keep in mind that we always only describe a small part of a complex pathophysiology. With our current data, we cannot determine whether carriers of DRB1*13 have a better or worse cardiovascular outcome and were only able to describe hints for a possible epidemiological relationship.

4.3 Phenotype of the autoimmune response

Another caveat in our study design is the inability to detect immune suppression in response to antigenic stimulation.

After stimulation and background-correction, some patients show a negative number of cytokine secreting cells. This seems biologically weird and might just be some expected technical variation. However, these negative numbers might as well represent Treg activity after stimulation with an autoantigen. While our study was not well designed to show this effect, it might be very relevant in the context of myocardial healing.

However, we detected no relevant secretion of cytokines typically secreted by Tregs such as IL-10, but measurement of a full cytokine panel was only performed in two MI patients showing a pro-inflammatory reaction. It might be possible that patients are separated, with some responding with an inflammatory reaction and some with a tolerant reaction after MI. This possibility could not have been detected in our study and would need further investigation.

Tregs were shown to be important mediators of healing after MI, mediating innate immune cell recruitment and scar formation.[52,54] The innate immune response to subsequent infarction after the first ischemic event was shown to be diminished. [72] This is in contrast to evidence for heightened remote immunity after previous tissue injury.[73] Adaptive mediation of specific immunity might play a role in mediation of these effects and induced Tregs could potentially diminish subsequent responses after the first MI, while not influencing remote reactions. There is potential for these Tregs to be long-lived, presenting a kind of memory for tissue injuries.

If this holds true, it would be very relevant to identify the status of tolerance in patients with cardiovascular disease to identify patients with a potentially deleterious course of disease.

4.4 Regulation and tolerance

The role of regulatory cells and establishment of tolerance still remains incompletely understood.

The immune system is a very delicate system, that affects virtually every condition in human health and disease. With modern ways of generating and analyzing vast amounts of data, we gather more and more insights on its inner workings. The frustrating situation in immunology still remains, that we know almost everything, but understand almost nothing. The very dangerous idea of breaching tolerance and allowing autoimmunity against self-antigens to restore balance after disbalancing injuries is an especially hard topic to investigate. As we were able to show autoimmunity against self in the context of myocardial infarction, the obvious question remains how the immune system manages to stop this reaction in time.

In models of autoimmune encephalomyelitis (EAE), an experimental model of multiple sclerosis, autoreactive $CD4^+$ T cells play an important role. Induction of EAE generates clonally expanded $CD4^+$ T cells, but these are kept in check by opposing mobilization of regulatory $CD8^+$ T cells.[74]

A similar mechanism might be envisioned for the regulation of cardiac autoreactivity after myocardial infarction. This bears great clinical relevance, as it is not uncommon for MI patients to suffer multiple ischemic events in their lives and the risk is enhanced following a first MI.[75] Opposing mechanisms to keep autoimmunity in check might be more pronounced in subsequent MI, impacting tissue injury and healing.

The autoimmune response we observed after myocardial infarction is interesting, because it does not follow the classical idea of tolerance against self and immune effects against foreign antigens. What we showed might be a prime example for “dynamic tolerance”, which describes regulatory capacities of the

immune system, which prevent autoimmune disease, but have to be dynamically adjusted to allow autoimmune reactions, when necessary.

One way for such a process to work would be, that autoimmune effector T cells are kept under control by opposing regulatory T cells. This could mean, that a network of T cells is needed to keep a stable balance between activation and suppression – in effect similar to the antibody network envisioned in the network hypothesis.[37] When there is a great stimulus and abundance of autoantigen, effector T cells would be stimulated. If the stimulus is high enough, T cells might get sufficiently activated to overcome suppression by opposing regulatory cells. The proliferation of effector cells would trigger expansion of the regulatory cells, which would finally be numerous enough to shut down the autoimmune response.

The threshold for autoimmunity is likely higher than the amount of antigen needed to trigger a pathogen-specific response. Autoantigens are always present in one's own body, thus always providing a small stimulus. This is especially tricky in a stressed organ like the heart, as there is evidence for cardiac cell damage in physiological situations like exercise.[76] This makes a constant activity of regulation necessary, providing an active threshold.

As pathogen-derived antigens are not present at baseline, this constant regulation is not necessary and likely not existent.

This concept is in line with previously described concepts. There could be a certain threshold for activation of the immune response, that is different for various immune settings. It would not be necessary to be generally tolerant against self, but tolerance could be dynamic and dependent on sensing of "danger".[40,41] The immune system as a whole would not be "active" or "in-active", but always in turmoil to conserve the organism.[77]

4.5 Interplay with atherosclerosis – chicken or the egg?

Myocardial infarction is classically preceded by atherosclerosis of the coronaries. The theory has long been pretty clear, that there are risk factors for

atherosclerosis, damage in the coronaries occurs and after time, devastation hits in the form of MI. Complications follow and patients are best cared for, but the main event was thought to have occurred.

In recent years, new insights complicated this straight causality. It was indeed shown that myocardial infarction accelerates atherosclerosis in the mouse model, making cardiovascular disease less of a one-way-street. This effect was shown to correlate with increased innate immune cell production, but an involvement of the adaptive immune system seems likely.[47] Clinical studies also showed an impact of high levels of physical activity on coronary artery calcification, suggesting a role of cardiac stress for the development of atherosclerosis.[78]

In a similar fashion, the immune reaction we observed might be not so straight after all. All patients in our MI group were selected for having an ischemic event, so our obvious idea was, that the death of cardiomyocytes has caused an inflammatory reaction.

The immune response we saw was mainly directed against ADRB1, which is expressed in cardiomyocytes. ADRB1 was also shown to be expressed in a variety of arteries, making an expression in the coronaries and a role in atherosclerosis possible.[79] This would provide an elegant explanation for the involvement of T cells in atherosclerosis, but awaits further confirmation.

The definition of MI indicates a pivotal role of plaque disruption in the pathogenesis. Plaque instability in atherosclerotic vessels can be caused by vascular smooth muscle cell (VSMC) apoptosis.[80] It has also been shown, that certain T cells induce VSMC apoptosis in the atherosclerotic plaque.[81] Further evidence exists for the relevance of monoclonal T cell populations in unstable angina, suggesting a role for antigen-specificity in plaque rupture.[82]

Apart from the autoantigen-specific T cells we observed after myocardial infarction, we also saw some hints for HLA-DRB1*13 carrier status being a risk factor for myocardial infarction. As the T cell response against ADRB1 was most

pronounced in patients with this specific HLA-genotype, a connection seems plausible. This challenges our belief of the MI being the trigger of an autoimmune reaction in our patients, as a higher risk for this event suggests some significant autoimmunity happening even before the development of ischemia.

An elegant explanation for this would be, that the immune response we observed is not caused by myocardial infarction, but rather the other way around. As atherosclerosis and plaque rupture are pivotal causes for the development of MI, it is possible that this vessel damage is partly caused or accelerated by an autoimmune reaction against ADRB1. The ADRB1 autoreactivity could be pronounced after myocardial infarction, because of the plaque rupture that precedes MI.

It will be interesting to further study this, as this might enable the measurement of an immune response leading to MI even before the event occurs.

There is a high risk for patients to develop a 2nd ischemic event after first MI – a clinical finding that is in line with the observed accelerated atherosclerosis after MI in the animal model. [47,75] Measuring ADRB1 directed autoimmunity after MI could uncover a basis for this observation and open diagnostic and therapeutic opportunities in the development of secondary MI.

4.6 Risk for myocardial infarction – potential clinical implications

Myocardial infarction is a devastating event in any one's life and much of our medical efforts is aimed at preventing this injury. To identify patients, where special prevention is beneficial, there is a substantial focus on risk factors for cardiovascular disease. Estimation of cardiovascular risk is an important part of individual therapeutic decisions reflected in current guidelines.[83,84] Although family history is recognized to be an important risk factor, genetic causes are still very heterogenous and genetic testing is not recommended to be used in clinical practice. Nonetheless, it would be very beneficial to identify patients with immunological predisposition for MI.

Quantification of cardiac autoimmunity might present a novel way to refine cardiovascular risk assessment and offer new opportunities for personalized medicine. As the autoimmune effects we showed are dependent on the patient's genetic HLA-group, it is likely to assume that the immune biology would vary between patients.

There is a constant search for precision treatments to better treat patients with heart disease. Current suggestions are focused on modulation of the immune system by modulating cytokines.[85] As cardiovascular diseases are not well enough understood, it is not yet possible to take precision further than that. If we advance our understanding, it might become possible to target not cytokines, but antigen-specific cells or antibodies, opening treatments as specific as vaccines.

While it has been some years, since the relevance of inflammation in heart failure was first recognized[86], many attempts to beneficially influence heart-directed inflammation failed.[87] Only recently was it possible to show a benefit for the modulation of inflammation in the context of heart failure in a randomized controlled trial.[88] Besides heart failure, there are also attempts to modulate the immune response after myocardial infarction to improve healing and protect healthy myocardium.[89,90] Immunosuppression with colchicine has been shown to be effective in reducing secondary ischemic events after myocardial infarction.[91] Pneumonia was a serious side effect of this treatment, highlighting the need for very specific immunomodulation to prevent unwanted side-effects of beneficial therapies.

We uncovered some interesting mechanism, which might predispose patients for atherosclerosis and ischemic events or modulate healing after myocardial infarction. This study was exploratory, and we included few patients, so clinical correlations cannot reliably be drawn from these results. To confirm our ideas and investigate possible predictive opportunities for patients, it will be necessary to continue our studies in a larger patient population. The autoantigen we

identified might present a unique way to stratify MI patients based on the immune response in a peri-infarct context. Whether this immune reaction might even be apparent before onset of MI and would thus predict ischemic events remains to be investigated.

5 References

1. Murphy, K. & Weaver, C. *Janeway's immunobiology*, (2017).
2. Medzhitov, R., Preston-Hurlburt, P. & Janeway, C.A. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* **388**, 394-397 (1997).
3. Gong, T., Liu, L., Jiang, W. & Zhou, R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nature Reviews Immunology* **20**, 95-112 (2020).
4. Orkin, S.H. & Zon, L.I. Hematopoiesis: An Evolving Paradigm for Stem Cell Biology. *Cell* **132**, 631-644 (2008).
5. Bassler, K., Schulte-Schrepping, J., Warnat-Herresthal, S., Aschenbrenner, A.C. & Schultze, J.L. The Myeloid Cell Compartment—Cell by Cell. *Annual Review of Immunology* **37**, 269-293 (2019).
6. Guermonprez, P., Valladeau, J., Zitvogel, L., Théry, C. & Amigorena, S. Antigen Presentation and T Cell Stimulation by Dendritic Cells. *Annual Review of Immunology* **20**, 621-667 (2002).
7. Ohnmacht, C.*, et al.* Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J Exp Med* **206**, 549-559 (2009).
8. Eriksson, U.*, et al.* Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat Med* **9**, 1484-1490 (2003).
9. Van der Borght, K.*, et al.* Myocardial Infarction Primes Autoreactive T Cells through Activation of Dendritic Cells. *Cell Rep* **18**, 3005-3017 (2017).
10. Mason, D. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunology Today* **19**, 395-404 (1998).
11. Wooldridge, L.*, et al.* A Single Autoimmune T Cell Receptor Recognizes More Than a Million Different Peptides *. *Journal of Biological Chemistry* **287**, 1168-1177 (2012).
12. Mestas, J. & Hughes, C.C. Of mice and not men: differences between mouse and human immunology. *J Immunol* **172**, 2731-2738 (2004).
13. Trowsdale, J. & Knight, J.C. Major Histocompatibility Complex Genomics and Human Disease. *Annual Review of Genomics and Human Genetics* **14**, 301-323 (2013).
14. Lederberg, J. JBS Haldane (1949) on infectious disease and evolution. *Genetics* **153**, 1-3 (1999).
15. Reusch, T.B.H., Häberli, M.A., Aeschlimann, P.B. & Milinski, M. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **414**, 300-302 (2001).
16. Evans, D.M.*, et al.* Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nature Genetics* **43**, 761-767 (2011).

17. Strange, A.*, et al.* A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nature Genetics* **42**, 985-990 (2010).
18. Shortman, K. & Wu, L. Early T Lymphocyte Progenitors. *Annual Review of Immunology* **14**, 29-47 (1996).
19. Goldrath, A.W. & Bevan, M.J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402**, 6-13 (1999).
20. Hunter, M.C., Teijeira, A. & Halin, C. T Cell Trafficking through Lymphatic Vessels. *Frontiers in Immunology* **7**(2016).
21. Zhu, J., Yamane, H. & Paul, W.E. Differentiation of Effector CD4 T Cell Populations. *Annual Review of Immunology* **28**, 445-489 (2010).
22. Vinuesa, C.G., Linterman, M.A., Yu, D. & MacLennan, I.C.M. Follicular Helper T Cells. *Annual Review of Immunology* **34**, 335-368 (2016).
23. Ruterbusch, M., Pruner, K.B., Shehata, L. & Pepper, M. In Vivo CD4+ T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. *Annual Review of Immunology* **38**, 705-725 (2020).
24. Eichmann, K.*, et al.* The network collective: Rise and fall of a scientific paradigm. *The Network Collective: Rise and Fall of a Scientific Paradigm*, 1-274 (2008).
25. Landsverk, O.J.B., Bakke, O. & Gregers, T.F. MHC II and the Endocytic Pathway: Regulation by Invariant Chain. *Scandinavian Journal of Immunology* **70**, 184-193 (2009).
26. Sette, A.*, et al.* Invariant chain peptides in most HLA-DR molecules of an antigen-processing mutant. *Science* **258**, 1801-1804 (1992).
27. Riberdy, J.M., Newcomb, J.R., Surman, M.J., Barbosat, J.A. & Cresswell, P. HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. *Nature* **360**, 474-477 (1992).
28. Busch, R.*, et al.* Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunological Reviews* **207**, 242-260 (2005).
29. Denzin, L.K. & Cresswell, P. HLA-DM induces clip dissociation from MHC class II α β dimers and facilitates peptide loading. *Cell* **82**, 155-165 (1995).
30. Sloan, V.S.*, et al.* Mediation by HLA-DM of dissociation of peptides from HLA-DR. *Nature* **375**, 802-806 (1995).
31. Sherman, M.A., Weber, D.A. & Jensen, P.E. Article. *Immunity* **3**, 197-205 (1995).
32. Blum, J.S., Wearsch, P.A. & Cresswell, P. Pathways of Antigen Processing. *Annual Review of Immunology* **31**, 443-473 (2013).
33. Sercarz, E.E. & Maverakis, E. MHC-guided processing: binding of large antigen fragments. *Nature Reviews Immunology* **3**, 621-629 (2003).
34. Blander, J.M. Phagocytosis and antigen presentation: a partnership initiated by Toll-like receptors. *Annals of the Rheumatic Diseases* **67**, iii44-iii49 (2008).
35. Akram, A. & Inman, R.D. Immunodominance: a pivotal principle in host response to viral infections. *Clin Immunol* **143**, 99-115 (2012).

36. Buus, S., Sette, A., Colon, S., Miles, C. & Grey, H. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* **235**, 1353-1358 (1987).
37. Jerne, N.K. Towards a network theory of the immune system. *Ann Immunol (Paris)* **125c**, 373-389 (1974).
38. Richter, P.H. A network theory of the immune system. *European Journal of Immunology* **5**, 350-354 (1975).
39. Jerne, N.K. Clonal selection in a lymphocyte network. *Soc Gen Physiol Ser* **29**, 39-48 (1974).
40. Matzinger, P. Tolerance, danger, and the extended family. *Annu Rev Immunol* **12**, 991-1045 (1994).
41. Matzinger, P. The Danger Model: A Renewed Sense of Self. *Science* **296**, 301-305 (2002).
42. Bowness, P. HLA B27 in health and disease: a double-edged sword? *Rheumatology* **41**, 857-868 (2002).
43. Rock, K.L. & Kono, H. The Inflammatory Response to Cell Death. *Annual Review of Pathology: Mechanisms of Disease* **3**, 99-126 (2008).
44. Roth, G.A.*, et al.* Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet* **392**, 1736-1788 (2018).
45. Swirski, F.K. & Nahrendorf, M. Cardioimmunology: the immune system in cardiac homeostasis and disease. *Nature Reviews Immunology* **18**, 733-744 (2018).
46. Hofmann, U. & Frantz, S. Role of T-cells in myocardial infarction. *European Heart Journal* **37**, 873-879 (2015).
47. Dutta, P.*, et al.* Myocardial infarction accelerates atherosclerosis. *Nature* **487**, 325-329 (2012).
48. Swirski, F.K.*, et al.* Identification of Splenic Reservoir Monocytes and Their Deployment to Inflammatory Sites. *Science* **325**, 612-616 (2009).
49. van der Laan, A.M.*, et al.* Monocyte subset accumulation in the human heart following acute myocardial infarction and the role of the spleen as monocyte reservoir. *Eur Heart J* **35**, 376-385 (2014).
50. Yan, X.*, et al.* Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol* **62**, 24-35 (2013).
51. Hofmann, U.*, et al.* Activation of CD4⁺ T Lymphocytes Improves Wound Healing and Survival After Experimental Myocardial Infarction in Mice. *Circulation* **125**, 1652-1663 (2012).
52. Weirather, J.*, et al.* Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res* **115**, 55-67 (2014).
53. Anzai, A.*, et al.* Regulatory Role of Dendritic Cells in Postinfarction Healing and Left Ventricular Remodeling. *Circulation* **125**, 1234-1245 (2012).
54. Rieckmann, M.*, et al.* Myocardial infarction triggers cardioprotective antigen-specific T helper cell responses. *J Clin Invest* **129**, 4922-4936 (2019).

55. Gil-Cruz, C.*, et al.* Microbiota-derived peptide mimics drive lethal inflammatory cardiomyopathy. *Science* **366**, 881-886 (2019).
56. Zal, B.*, et al.* Heat-Shock Protein 60-Reactive CD4⁺CD28^{null} T Cells in Patients With Acute Coronary Syndromes. *Circulation* **109**, 1230-1235 (2004).
57. Created with BioRender.com.
58. Uhlen, M.*, et al.* Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).
59. Uhlen, M.*, et al.* Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* **28**, 1248-1250 (2010).
60. UniProt, C. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* **47**, D506-D515 (2019).
61. Greenbaum, J.*, et al.* Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. *Immunogenetics* **63**, 325-335 (2011).
62. Wang, P.*, et al.* A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLoS Comput Biol* **4**, e1000048 (2008).
63. Wang, P.*, et al.* Peptide binding predictions for HLA DR, DP and DQ molecules. *BMC Bioinformatics* **11**, 568 (2010).
64. Song, J.*, et al.* PROSPER: an integrated feature-based tool for predicting protease substrate cleavage sites. *PLoS One* **7**, e50300 (2012).
65. Ramachandran, H.*, et al.* Optimal thawing of cryopreserved peripheral blood mononuclear cells for use in high-throughput human immune monitoring studies. *Cells* **1**, 313-324 (2012).
66. IPD-IMGTA/HLA, https://www.ebi.ac.uk/ipd/imgt/hla/about/statistics/.
67. Laydon, D.J., Bangham, C.R.M. & Asquith, B. Estimating T-cell repertoire diversity: limitations of classical estimators and a new approach. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**, 20140291 (2015).
68. Jahns, R., Boivin, V. & Lohse, M.J. Beta 1-adrenergic receptor-directed autoimmunity as a cause of dilated cardiomyopathy in rats. *Int J Cardiol* **112**, 7-14 (2006).
69. Störk, S.*, et al.* Stimulating autoantibodies directed against the cardiac beta1-adrenergic receptor predict increased mortality in idiopathic cardiomyopathy. *Am Heart J* **152**, 697-704 (2006).
70. Paakkanen, R.*, et al.* Proinflammatory HLA-DRB1*01-haplotype predisposes to ST-elevation myocardial infarction. *Atherosclerosis* **221**, 461-466 (2012).
71. Oka, S.*, et al.* Protective Effect of the HLA-DRB1*13:02 Allele in Japanese Rheumatoid Arthritis Patients. *PLOS ONE* **9**, e99453 (2014).
72. Cremer, S.*, et al.* Diminished Reactive Hematopoiesis and Cardiac Inflammation in a Mouse Model of Recurrent Myocardial Infarction. *J Am Coll Cardiol* **75**, 901-915 (2020).

73. Hoyer, F.F.*, et al.* Tissue-Specific Macrophage Responses to Remote Injury Impact the Outcome of Subsequent Local Immune Challenge. *Immunity* **51**, 899-914.e897 (2019).
74. Saligrama, N.*, et al.* Opposing T cell responses in experimental autoimmune encephalomyelitis. *Nature* **572**, 481-487 (2019).
75. Jernberg, T.*, et al.* Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective. *European Heart Journal* **36**, 1163-1170 (2015).
76. Marshall, L.*, et al.* Effect of Exercise Intensity and Duration on Cardiac Troponin Release. *Circulation* **141**, 83-85 (2020).
77. Vaz, N.M., Ramos, G.C., Pordeus, V. & Carvalho, C.R. The conservative physiology of the immune system. A non-metaphoric approach to immunological activity. *Clin Dev Immunol* **13**, 133-142 (2006).
78. DeFina, L.F.*, et al.* Association of All-Cause and Cardiovascular Mortality With High Levels of Physical Activity and Concurrent Coronary Artery Calcification. *JAMA Cardiology* **4**, 174-181 (2019).
79. Chruscinski, A.*, et al.* Differential Distribution of β-Adrenergic Receptor Subtypes in Blood Vessels of Knockout Mice Lacking $\beta 1$ - or $\beta 2$-Adrenergic Receptors. *Molecular pharmacology* **60**, 955-962 (2001).
80. Clarke, M.C.H.*, et al.* Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nature Medicine* **12**, 1075-1080 (2006).
81. Sato, K.*, et al.* TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. *J Exp Med* **203**, 239-250 (2006).
82. Liuzzo, G.*, et al.* Monoclonal T-Cell Proliferation and Plaque Instability in Acute Coronary Syndromes. *Circulation* **101**, 2883-2888 (2000).
83. Arnett, D.K.*, et al.* 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease. *Journal of the American College of Cardiology* **74**, e177-e232 (2019).
84. Piepoli, M.F.*, et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *European Heart Journal* **37**, 2315-2381 (2016).
85. Crea, F. & Liuzzo, G. Anti-inflammatory treatment of acute coronary syndromes: the need for precision medicine. *European Heart Journal* **37**, 2414-2416 (2016).
86. Levine, B., Kalman, J., Mayer, L., Fillit, H.M. & Packer, M. Elevated Circulating Levels of Tumor Necrosis Factor in Severe Chronic Heart Failure. *New England Journal of Medicine* **323**, 236-241 (1990).
87. Adamo, L., Rocha-Resende, C., Prabhu, S.D. & Mann, D.L. Reappraising the role of inflammation in heart failure. *Nature Reviews Cardiology* **17**, 269-285 (2020).

88. Everett, B.M.*, et al.* Anti-Inflammatory Therapy With Canakinumab for the Prevention of Hospitalization for Heart Failure. *Circulation* **139**, 1289-1299 (2019).
89. Broch, K.*, et al.* Randomized Trial of Interleukin-6 Receptor Inhibition in Patients With Acute ST-Segment Elevation Myocardial Infarction. *J Am Coll Cardiol* **77**, 1845-1855 (2021).
90. Kleveland, O.*, et al.* Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. *Eur Heart J* **37**, 2406-2413 (2016).
91. Tardif, J.-C.*, et al.* Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *New England Journal of Medicine* **381**, 2497-2505 (2019).

Supplementary Material 1:

Cardiac Antigen Atlas

Volume II: Class-II-restricted, Human

Immunocardiology Lab

Nils Hapke, Chiara Gaal, Gustavo Ramos

Deutsches Zentrum für Herzinsuffizienz

Universitätsklinikum Würzburg

Dec 2017

Preamble: Classes of Antigens that could be relevant in the MI context:

- **i "Cardiac antigens":** Heart-specific proteins released after cardiomyocyte necrosis (mainly motor proteins)
- **iii Stress-related antigens:** Antigens related to tissue injury whether or not in the myocardial tissue (e.g. Heat-Shock Proteins)
- **iii Thrombus-derived antigens:** e.g. fibriogens
-
- **iv Modified proteins ("Altered-self"):** Proteins modified in the ischemic milieu (e.g. Citrullinated Fibrinogen)

i. Cardiac antigens and the Cardiac Proteome

A transcriptome analysis showed that 63% of all human proteins (n=19628) are expressed in the heart. Of note, **201** of these genes showed an elevated expression in heart, as compared to other tissue types. An analysis of genes with elevated expression in heart reveals that a majority of the corresponding proteins are expressed in the cytoplasm, in different regions of the sarcomeres, with functions related to muscle contraction, ion transport and ATPase activity. Further analyses showed that 82% of the mRNA molecules derived from heart tissue correspond to housekeeping genes and only 13% of the mRNA pool corresponds to genes categorized to be either heart enriched, group enriched or, heart enhanced (Source: The Human Protein Atlas, http://www.proteinatlas.org/humanproteome/heart).

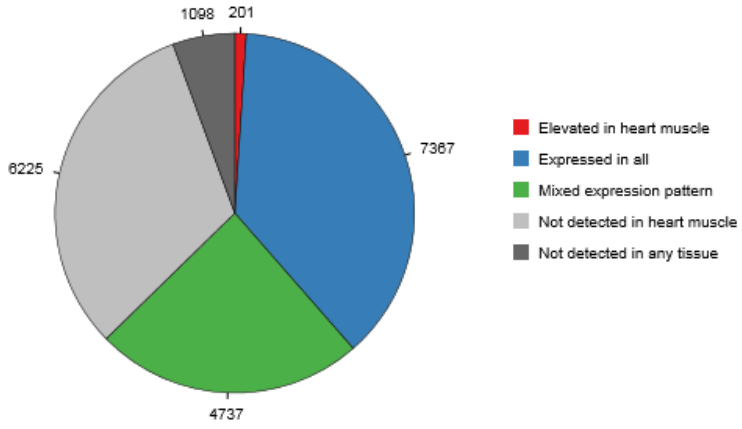

Table I: Groups of proteins preferentially expressed in the heart.

Category	Number of genes	Description
Tissue enriched	28	At least five-fold higher mRNA levels in a particular tissue as compared to all other tissues
Group enriched	92	At least five-fold higher mRNA levels in a group of 2-7 tissues
Tissue enhanced	81	At least five-fold higher mRNA levels in a particular tissue as compared to average levels in all tissues

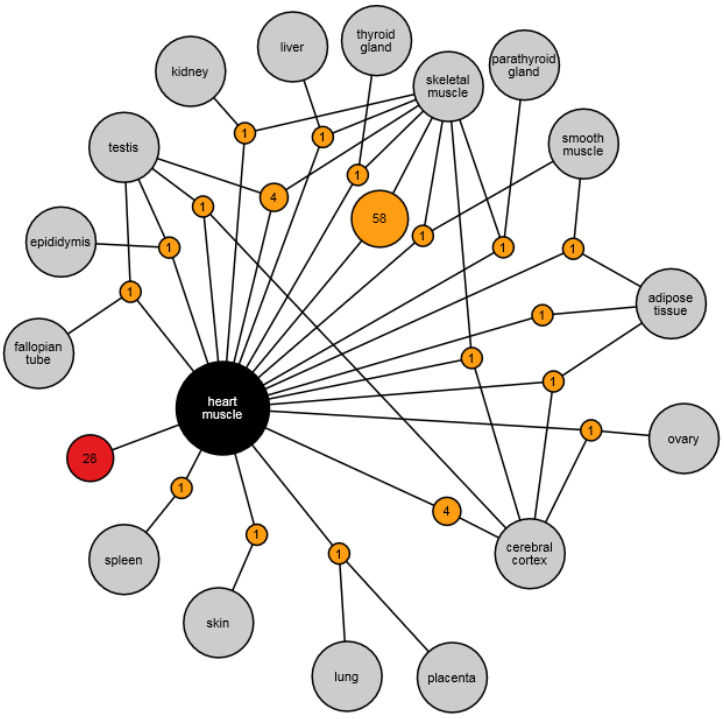

Figure 2: An interactive network plot of the heart muscle enriched and group enriched genes connected to their respective enriched tissues (grey circles). Red nodes represent the number of heart muscle enriched genes and orange nodes represent the number of genes that are group enriched. The sizes of the red and orange nodes are related to the number of genes displayed within the node. Each node is clickable and results in a list of all enriched genes connected to the highlighted edges. The network is limited to group enriched genes in combinations of up to 3 tissues, but the resulting lists show the complete set of group enriched genes in the particular tissue

Table II. The 12 genes with the highest level of enriched expression in the heart.

Gene	Description	Predicted localization	mRNA (tissue)	TS-score
NPPB	natriuretic peptide B	Secreted	2863.6	1602
MYL7	myosin, light chain 7, regulatory	Intracellular	9298.4	1460
NPPA	natriuretic peptide A	Intracellular,Secreted	26555.9	1175
MYBPC3	myosin binding protein C, cardiac	Intracellular	684.8	472
TNNT2	troponin T type 2 (cardiac)	Intracellular	5036.0	199
BMP10	bone morphogenetic protein 10	Secreted	612.5	171
TNNI3	troponin I type 3 (cardiac)	Intracellular	3237.3	158
MYH6	myosin, heavy chain 6, cardiac muscle, alpha	Intracellular	2135.5	111
ANKRD1	ankyrin repeat domain 1 (cardiac muscle)	Intracellular	3570.9	104
RD3L	retinal degeneration 3-like	Intracellular	21.0	91
SBK2	SH3 domain binding kinase family, member 2	Membrane	26.5	33
MYL4	myosin, light chain 4, alkali; atrial, embryonic	Intracellular	2225.0	28

ii Stress-related antigens:

Heat-Shock Proteins (HSP) are ubiquitously expressed in several tissues, and these proteins have been identified as a family of stress-induced proteins. HSPs are also important players in the control of the immune responses. These inflammation-associated proteins are targeted by HSP-specific T cells and antibodies in healthy subjects and also during the course of autoimmune disorders. We previously found that the injured myocardium up-regulates the expression of Hsp72 and that this protein is targeted by HSP72-specific antibodies. Furthermore, it has been demonstrated that HSP60 and HSP70-specific immune responses participate in the pathogenesis of atherosclerosis. Therefore, it may be also worth considering that MI would trigger HSP-specific T-cell responses.

iii Thrombus-derived Antigens:

The infarcted myocardium also harbors many cell types and proteins that are normally not present in the healthy tissue (e.g. proteins form the complement and coagulation cascade). Microthrombi are often found spread throughout the infarcted myocardium. These fibrin-rich clots are, to a large extent, cleared by phagocytes which can also present class-II restricted antigens to CD4+T-cells. Therefore it is reasonable to assume that, beyond heart-specific antigens, other epitopes derived from the clotting cascade could also contribute to the post-MI T-cell responses.

iv. **"Altered-self"**

MI leads to a strong local inflammatory reaction, and oxidative stress that could ultimately cause to protein modifications. Thus, beyond the cardiac antigens, it is also worth considering that proteins modified in the injured myocardium could be also a source of antigenic stimulation after MI. It has been reported that proteins modified in the inflammatory context (altered-self) can be a relevant source of autoantigens and participate in the genesis of some autoimmune diseases. A key example of this phenomenon is the conversion of arginine residues in proteins into its polar analogue citrulline by a group of enzymes termed as peptidyl arginine deiminase (PAD). Protein citrullination has been shown to greatly enhance immune recognition of joint-associated proteins, which are selectively targeted by autoreactive T and B cells in patients with rheumatoid arthritis. PAD enzymes are mainly expressed by neutrophils, which are a preponderant cell type infiltrating the ischemic myocardium. In fact, a recent study found that some MI patients develop anti-Citrullin antibodies, and that this correlated with higher mortality (Hermans, et al., 2017). Therefore, we hypothesize that proteins citrullinated in the ischemic myocardium would be also an important source of MHC-II restricted antigens (e.g. citrullinated Myosin, etc).

Last but not least, one should also consider that blood-born proteins modified in the myocardial milieu could be also relevant in this process. It has been shown that **citrullinated fibrinogen** is an important antigen in rheumatoid arthritis. Thus, considering that thrombi formation is important in the pathogenesis of MI, this would be also a relevant target to be considered.

Method

1. **Define potentially relevant proteins in each of the above-mentioned categories and gather basic biochemical information**

    a. **Protein description:**

        i. Acquire information about the protein FASTA sequence, mass, size on (Uniprot.org);
        ii. Acquire information about tissue distribution and expression levels in the heart (Proteinatlas.org)

2. **Epitope prediction (MHC-II binding peptides)** (http://tools.iedb.org/main/)

    a. T-cell prediction tools > Peptide binding to MHC-II molecules. Select peptides below the 10th percentile.
        i. For mouse: H2-IAd (Balb/c mouse strain) and H2-IAb (C56BL/6 strain)
        ii. For Human: select peptides based on the HLADRB1*001:001 allele, as this is expressed by the NSG-DR1 mouse strain. Other DR1 alleles may be eventually tested afterwards.

    b. **In silico prediction of MHC-II processing**
        i. Proteases cleavage site-prediction: Go to **https://prosper.erc.monash.edu.au/** , type in the FASTA sequence, file name and email. A map of all protease cleaving sites within the protein will be reported. This approach will indicate whether other proteases present in the infarcted myocardium (e.g. MMP2, MMP9) can cleave an epitope of interest.

    c. Result: epitope of interest = binds with mid-high affinity to a particular MHC-II (below 10th percentile), not targeted by cathepsins B, D, S, L.

3. **Check the catalog of annotated peptides already proven to bind to the MHC-II of interest**

    a. The IEDB, www.iedb.org, contains information on immune epitopes—the molecular targets of adaptive immune responses—curated from the published literature and submitted by National Institutes of Health funded epitope discovery efforts. From 2004 to 2012 the IEDB curation of journal articles published since 1960 has caught up to the present day, with >95% of relevant published literature manually curated amounting to more than 15 000 journal articles and more than 704 000 experiments to date. The following criteria was taken into consideration for including additional peptides:

        i. Annotated peptides already used in CD4 T-cell functional assays
        ii. Annotated peptides found in MHC-II-elution screenings

Proteins of Interest

Table III. List of proteins that will be focused in this document

Heart-specific Antigens			
Protein	**Cardiac exp. level**	**Tissue distribution**	**Localization**
Actc1 Actin alpha 1, cardiac isoform	High	Heart-enriched	Intracellular (motor)
Adrb1 Adrenoceptor Beta 1	High	Tissue-enhanced	Membrane
Mybpc3 Myosin binding protein C, cardiac	High	Heart-enriched	Intracellular (motor)
Myh6 Myosin Heavy Chain 6, cardiac isoform	High	Heart-enriched	Intracellular (motor)
Mhl2 Myosin light Chain 2 (Ventricular)	High	Heart-enriched	Intracellular (motor)
Tnni3 Troponin I type3, cardiac isoform	High	Heart-enriched	Intracellular (motor)
Tnnt2 Troponin T type2, cardiac isoform	High	Heart-enriched	Intracellular (motor)
Inflammation-associated-Antigens			
Hspb3 Heat-Shock Protein 27	High (RNA)	Heart-enriched	Intracellular
Hspa1a Heat-Shock Protein 72	Medium (steady-state) High upon disease	Mixed, associated with inflammation	Mitochondrial
Clot-derived-Antigens			
Fga Fibrinogen-alpha chain	Not expressed; fibrin deposited in the injured heart	Liver	Secreted (Circulation)
Fgb Fibrinogen-beta chain	Same as Fga	Liver	Secreted (Circulation)
Fgg Fibrinogen-gamma chain	Same as Fga	Liver	Secreted (Circulation)
Modified Proteins			
Citrullinated Fibrinogen	To be defined	Expressed in liver, secreted into circulation,	Modified in the infarcted myocardium (?)

Human MHC-II-restricted Antigens

i Cardiac antigens

ACTC1 …….
ADRB1 ……..
MYBPC3 …….
MYH6 ……..
MHL2 ……..
TNNI3 ……..

ii Stress-related antigens

HSPB3 …….
HSP70 ……..

iii Clot-derived antigens
FGA ……..
FGB ……..
FGG ……..

iv Modified proteins
CIT FGA ……

Peptide Library ……..……

Protein name:	**ACTC1 – Actin, alpha 1, cardiac isoform**
Species:	*Homo sapiens*
Mass/Length	42 kDa/ 377 aa
FASTA Sequence : Source: Uniprot Date: 28.12.2017	MCDDEETTALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPR HQGVMVGMGQKDSYVGDEAQSKRGILTLKYPIEHGIITWDD MEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREKMTQIM FETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIY EGYALPHAIMRLDLAGRDLTDYLMKILTERGYSFVTTAEREIV RDIKEKLCYVALDFENEMATAASSSSLEKSYELPDGQVITIGN ERFRCPETLFQPSFIGMESAGIHETTYNSIMKCDIDIRKDLYA NNVLSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYS VWIGGSILASLSTFQQMWISKQEYDEAGPSIVHRKCF
Expression Pattern: Source: Human Protein Atlas Date: 28.12.2017	Selective cytoplasmic expression in heart and skeletal muscle.

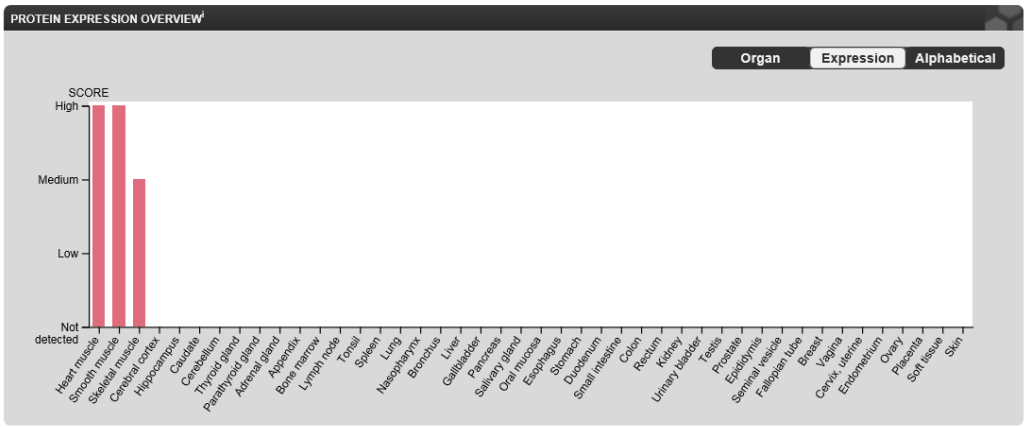

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas: Proteinatlas.org)

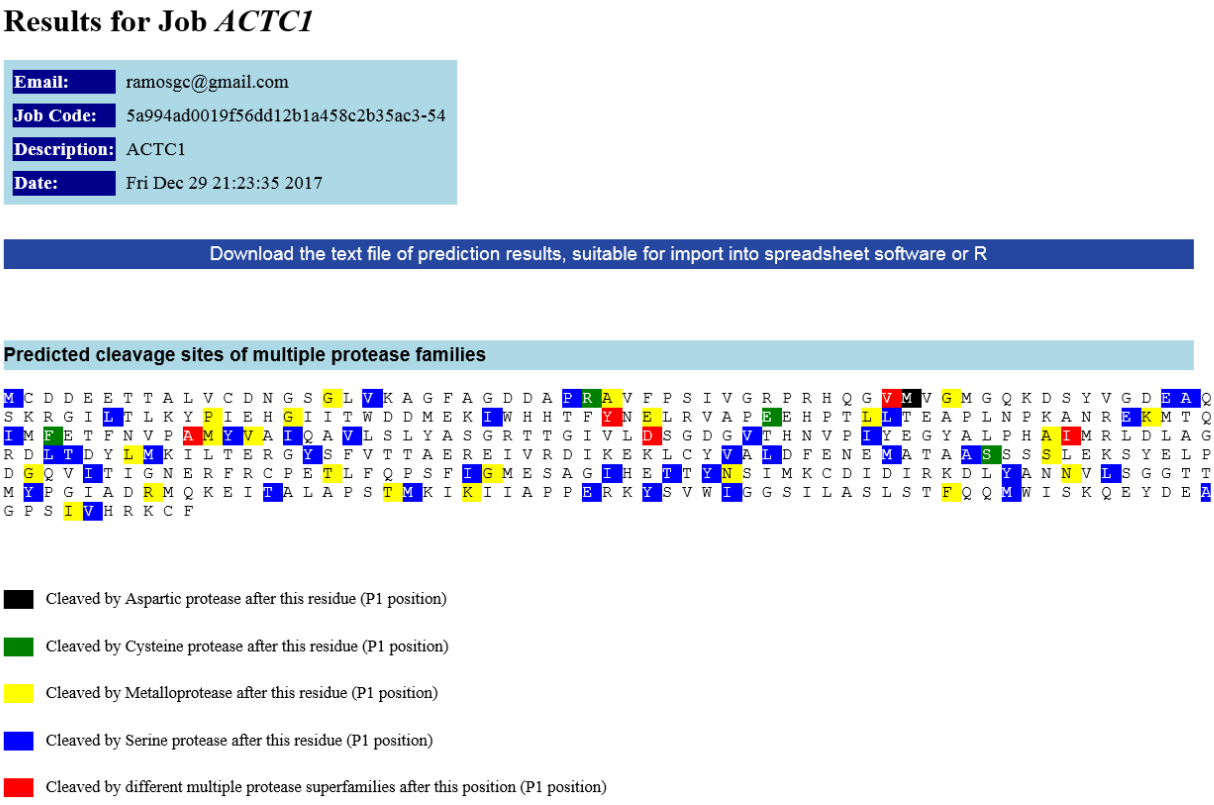

Figure 2: Proteases cleaving sites (Source: Prosper: https://prosper.erc.monash.edu.au/)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=9590327a940d1451451690b9aa14015e-32

Table I: MHC-II prediction (Source: IEDB: http://tools.immuneepitope.org/mhcii/)

	DRB1*01*01
Number of peptides < 10 Percentile Rank	24
Number of Peptide cores with high affinity (< Ic50 50 nM)	24
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide Position	Peptide Sequence	Percentile
DRB1*01*01	ACTC1$_{132\text{-}146}$	PA**MYVAIQAVL**SLYA	0.76
DRB1*01*01	ACTC1$_{137\text{-}150}$	AIQA**VLSLYASGR**TT	7.08
DRB1*01*01	ACTC1$_{265\text{-}279}$	QPS**FIGMESAG**IHET	0.97
DRB1*01*01	ACTC1$_{316\text{-}330}$	QKE**ITALAPST**MKIK	4.16

Protein name:	**Adrb1 (Adrenoceptor Beta 1)**
Species:	*Homo sapiens*
Mass/Length	51,323 kDa/477 aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MGAGVLVLGASEPGNLSSAAPLPDGAATAARLLVPASPP ASLLPPASESPEPLSQQWTAGMGLLMALIVLLIVAGNVLV IVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATIVV WGRWEYGSFFCELWTSVDVLCVTASIETLCVIALDRYLAI TSPFRYQSLLTRARARGLVCTVWAISALVSFLPILMHWW RAESDEARRCYNDPKCCDFVTNRAYAIASSVVSFYVPLC IMAFVYLRVFREAQKQVKKIDSCERRFLGGPARPPSPSP SPVPAPAPPPGPPRPAAAAATAPLANGRAGKRRPSRLV ALREQKALKTLGIIMGVFTLCWLPFFLANVVKAFHRELVP DRLFVFFNWLGYANSAFNPIIYCRSPDFRKAFQRLLCCA RRAARRRHATHGDRPRASGCLARPGPPPSPGAASDDD DDDVVGATPPARLLEPWAGCNGGAAADSDSSLDEPCR PGFASESKV
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Cytoplasmic expression in heart myocytes, glial cells and a subset of placental trophoblastic cells. Predicted Membrane Proteine.

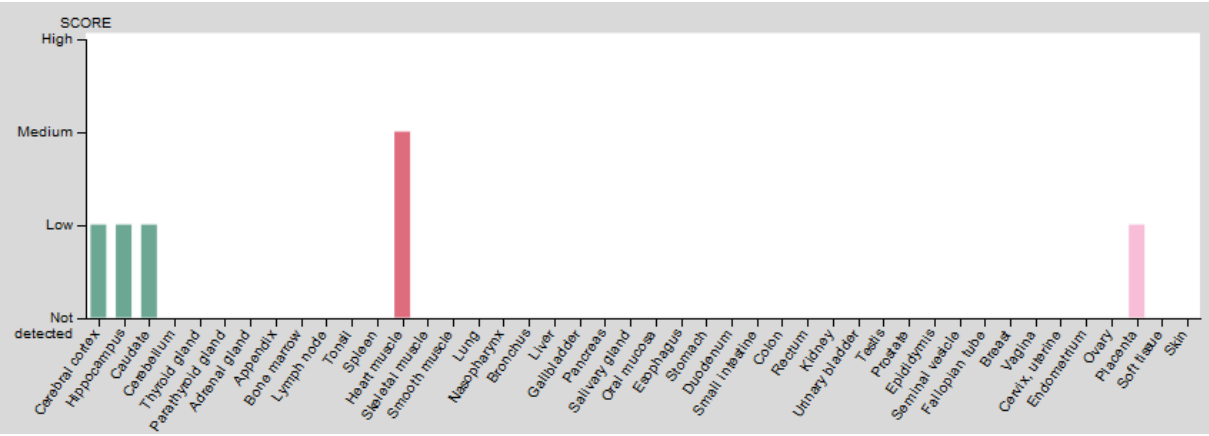

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas)

Results for Job *Adrb1*

Email:	hapke_n@ukw.de
Job Code:	d646980467859a9a83455fbd3afb63ab-51
Description:	Adrb1
Date:	Tue Dec 12 00:51:23 2017

Download the text file of prediction results, suitable for import into spreadsheet software or R

Predicted cleavage sites of multiple protease families

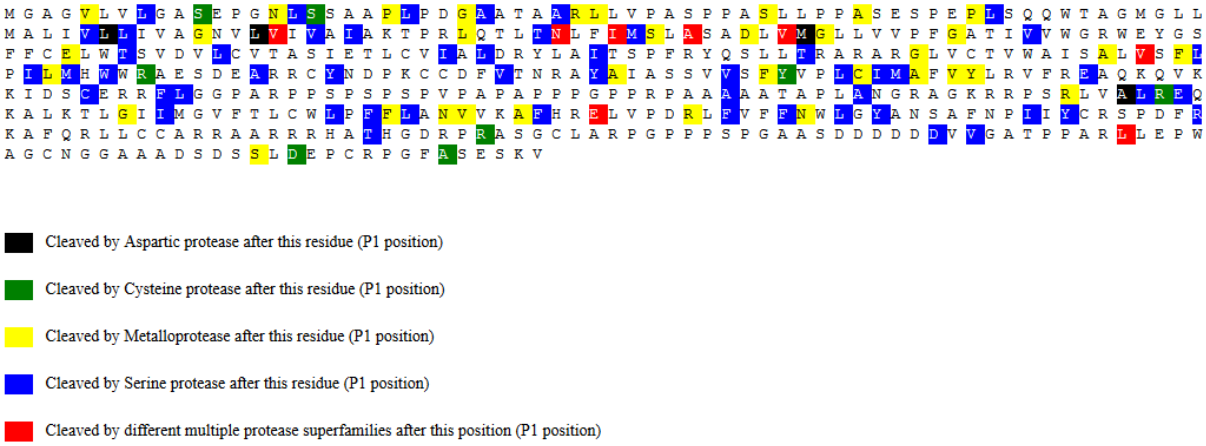

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=d646980467859a9a83455fbd3afb63ab-51

Table I: MHC-II prediction (Source: IEDB)

	DRB1*01*01
Number of peptides < 10 Percentile Rank	68
Number of Peptide cores with high affinity (< Ic50 50 nM)	68
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0

Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide position	Peptide Sequence	Percentile
DRB1*01*01	ADRB1$_{28-44}$	TA**ARLLVPASP**PASLLP	5.46
DRB1*01*01	ADRB1$_{63-77}$	**LLMALIVLL**IVAGNV	9.01
DRB1*01*01	ADRB1$_{67-81}$	LIV**LLIVAGNVL**VIV	1.36
DRB1*01*01	ADRB1$_{75-89}$	**GNVLVIVAI**AKTPRL	8.28
DRB1*01*01	ADRB1$_{76-90}$	NV**LVIVAIAKT**PRLQ	9.87
DRB1*01*01	ADRB1$_{92-106}$	**LTNLFIMSL**ASADLV	0.88
DRB1*01*01	ADRB1$_{93-107}$	TN**LFIMSLASA**DLVM	0.77
DRB1*01*01	ADRB1$_{96-110}$	F**IMSLASADL**VMGLL	3.24
DRB1*01*01	ADRB1$_{97-111}$	IMSLAS**ADLVMGLLV**	5.59
DRB1*01*01	*ADRB1$_{107-121}$	MGL**LVVPFGATI**VVW	7.51
DRB1*01*01	ADRB1$_{153-167}$	ALD**RYLAITSPF**RYQ	6.65
DRB1*01*01	ADRB1$_{164-178}$	FR**YQSLLTRAR**ARGL	4.95
DRB1*01*01	ADRB1$_{167-181}$	**QSLLTRARA**RGLVCT	7.48
DRB1*01*01	ADRB1$_{168-182}$	SL**LTRARARGL**VCTV	8.87
DRB1*01*01	ADRB1$_{177-191}$	GLV**CTVWAISAL**VSF	8.67

DRB1*01*01	ADRB1$_{215-229}$	CCD**FVTNRAYAI**ASS	0.62
DRB1*01*01	ADRB1$_{220-234}$	**TNRAYAIAS**SVVSFY	3.24
DRB1*01*01	ADRB1$_{221-235}$	NRAYAI**ASSVVSFYV**	3.02
DRB1*01*01	ADRB1$_{311-325}$	**PSRLVALRE**QKALKT	6.00
DRB1*01*01	ADRB1$_{312-326}$	SRL**VALREQKAL**KTL	5.59
DRB1*01*01	ADRB1$_{334-348}$	TLCWLP**FFLANVVKA**	8.67
DRB1*01*01	ADRB1$_{335-349}$	LCWLPF**FLANVVKAF**	6.52

* Experimental evidence

Protein name:	**MYBPC3, myosin binding protein C, cardiac**
Species:	*Homo sapiens*
Mass/Length	140.762 kDa/1,274 aa
FASTA Sequence : Source: Uniprot Date: 10.11.2017	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAG VKVRWQRGGSDISASNKYGLATEGTRHTLTVREVGPA DQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEA TGAPGEAPAPAAELGESAPSPKGSSSAALNGPTPGAP DDPIGLFVMRPQDGEVTVGGSITFSARVAGASLLKPPV VKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHI TDAQPAFTGSYRCEVSTKDKFDCSNFNLTVHEAMGTG DLDLLSAFRRTSLAGGGRRISDSHEDTGILDFSSLLKKR DSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQY GVTDLRGMLKRLKGMRRDEKKSTAFQKKLEPAYQVSK GHKIRLTVELADHDAEVKWLKNGQEIQMSGSKYIFESIG AKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPV LITRPLEDQLVMVGQRVEFECEVSEEGAQVKWLKDGV ELTREETFKYRFKKDGQRHHLIINEAMLEDAGHYALCTS GGQALAELIVQEKKLEVYQSIADLMVGAKDQAVFKCEV SDENVRGVWLKNGKELVPDSRIKVSHIGRVHKLTIDDVT PADEADYSFVPEGFACNLSAKLHFMEVKIDFVPRQEPP KIHLDCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIWQK AITQGNKAPARPAPDAPEDTGDSDEWVFDKKLLCETEG RVRVETTKDRSIFTVEGAEKEDEGVYTVTVKNPVGEDQ VNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPAYDG GQPILGYILERKKKKSYRWMRLNFDLIQELSHEARRMIE GVVYEMRVYAVNAIGMSRPSPASQPFMPIGPPSEPTHL AVEDVSDTTVSLKWRPPERVGAGGLDGYSVEYCPEGC SEWVAALQGLTEHTSILVKDLPTGARLLFRVRAHNMAG PGAPVTTTEPVTVQEILQRPRLQLPRHLRQTIQKKVGEP VNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRNSPTDTI LFIRAARRVHSGTYQVTVRIENMEDKATLVLQVVDKPSP PQDLRVTDAWGLNVALEWKPPQDVGNTELWGYTVQK ADKKTMEWFTVLEHYRRTHCVVPELIIGNGYYFRVFSQ NMVGFSDRAATTKEPVFIPRPGITYEPPNYKALDFSEAP SFTQPLVNRSVIAGYTAMLCCAVRGSPKPKISWFKNGL DLGEDARFRMFSKQGVLTLEIRKPCPFDGGIYVCRATN LQGEARCECRLEVRVPQ
Expression Pattern: Source: Human Protein Atlas	Highly selective cytoplasmic expression in heart myocytes.

Date: 10.11.2017

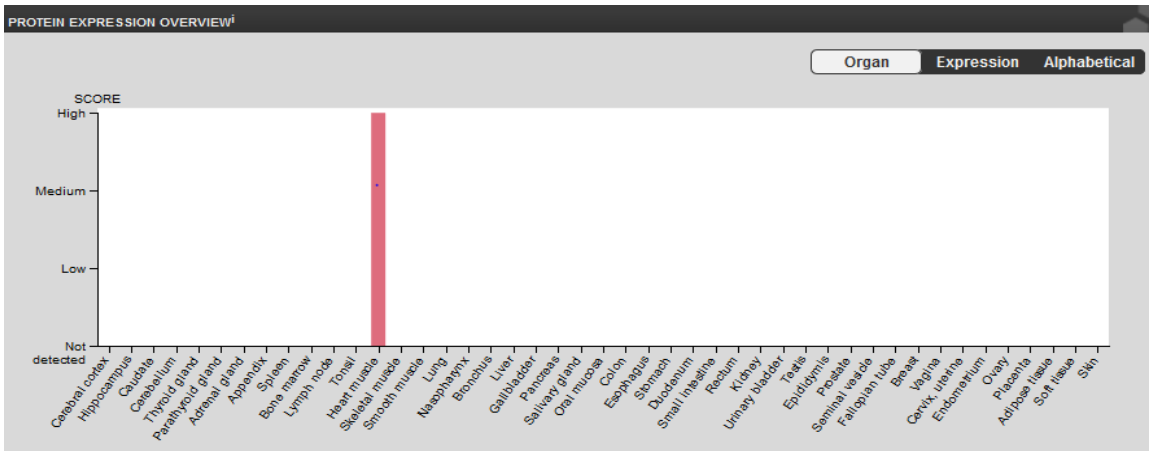

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas: Proteinatlas.org)

Results for Job *Mybpc3*

Email:	hapke_n@ukw.de
Job Code:	d49bb43f5ecb1540c7f2f651608f0af5-27
Description:	Mybpc3
Date:	Mon Dec 11 23:32:59 2017

Download the text file of prediction results, suitable for import into spreadsheet software or R

Predicted cleavage sites of multiple protease families

Cleaved by Aspartic protease after this residue (P1 position)

Cleaved by Cysteine protease after this residue (P1 position)

Cleaved by Metalloprotease after this residue (P1 position)

Cleaved by Serine protease after this residue (P1 position)

Cleaved by different multiple protease superfamilies after this position (P1 position)

Figure 2: Proteases cleaving sites (Source: Prosper: https://prosper.erc.monash.edu.au/)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=d49bb43f5ecb1540c7f2f651608f0af5-27

Table I: MHC-II prediction (Source: IEDB: http://tools.immuneepitope.org/mhcii/)

	DRB1*01*01
Number of peptides < 10 Percentile Rank	50
Number of Peptide cores with high affinity (< Ic50 50 nM)	50
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide core	Peptide position	Percentile
DRB1*01*01	MYBPC3$_{76-90}$	QG**SYAVIAGSS**KVKF	6.87
DRB1*01*01	MYBPC3$_{171-185}$	SIT**FSARVAGAS**LLK	6.46
DRB1*01*01	MYBPC3$_{346-360}$	RGM**LKRLKGMRR**DEK	3.95
DRB1*01*01	MYBPC3$_{409-423}$	**KYIFESIGA**KRTLTI	1.15
DRB1*01*01	MYBPC3$_{410-424}$	Y**IFESIGAKR**TLTIS	1.36
DRB1*01*01	MYBPC3$_{412-426}$	FESIGA**KRTLTISQC**	5.79
DRB1*01*01	MYBPC3$_{444-458}$	STE**LFVKEPPVL**ITR	2.27
DRB1*01*01	MYBPC3$_{544-558}$	**KLEVYQSIA**DLMVGA	5.95
DRB1*01*01	MYBPC3$_{545-559}$	LEVYQS**IADLMVGAK**	5.43

DRB1*01*01	MYBPC3$_{618-632}$	PEG**FACNLSAKL**HFM	7.88
DRB1*01*01	MYBPC3$_{656-670}$	PDT**IVVVAGNKL**RLD	3.71
DRB1*01*01	MYBPC3$_{936-950}$	TG**ARLLFRVRA**HNMA	9.06
DRB1*01*01	MYBPC3$_{1023-1037}$	NSPT**DTILFIRAA**RR	7.08
DRB1*01*01	MYBPC3$_{1026-1040}$	TDTI**LFIRAARRV**HS	4.72
DRB1*01*01	MYBPC3$_{1134-1148}$	GYY**FRVFSQNMV**GFS	5.59
DRB1*01*01	MYBPC3$_{1224-1238}$	DARFRM**FSKQGVLTL**	8.09

Protein name:	**Myh6 – Myosin, heavy chain 6, cardiac muscle, alpha**
Species:	*Homo sapiens*
Mass/Length	223.735 kDa/1,939 aa (whole protein)
FASTA Sequence : Source: Uniprot Date: 05.12.2017	MTDAQMADFGAAAQYLRKSEKERLEAQTRPFDIRTECFVPDDKEEFVKAKIL SREGGKVIAETENGKTVTVKEDQVLQQNPPKFDKIEDMAMLTFLHEPAVLFNL KERYAAWMIYTYSGLFCVTVNPYKWLPVYNAEVVAAYRGKKRSEAPPHIFSIS DNAYQYMLTDRENQSILITGESGAGKTVNTKRVIQYFASIAAIGDRGKKDNAN ANKGTLEDQIIQANPALEAFGNAKTVRNDNSSRFGKFIRIHFGATGKLASADIE TYLLEKSRVIFQLKAERNYHIFYQILSNKKPELLDMLLVTNNPYDYAFVSQGEV SVASIDDSEELMATDSAFDVLGFTSEEKAGVYKLTGAIMHYGNMKFKQKQRE EQAEPDGTEDADKSAYLMGLNSADLLKGLCHPRVKVGNEYVTKGQSVQQVY YSIGALAKAVYEKMFNWMVTRINATLETKQPRQYFIGVLDIAGFEIFDFNSFEQ LCINFTNEKLQQFFNHHMFVLEQEEYKKEGIEWTFIDFGMDLQACIDLIEKPM GIMSILEEECMFPKATDMTFKAKLYDNHLGKSNNFQKPRNIKGKQEAHFSLIH YAGTVDYNILGWLEKNKDPLNETVVALYQKSSLKLMATLFSSYATADTGDSG KSKGGKKKGSSFQTVSALHRENLNKLMTNLRTTHPHFVRCIIPNERKAPGVM DNPLVMHQLRCNGVLEGIRICRKGFPNRILYGDFRQRYRILNPVAIPEGQFIDS RKGTEKLLSSLDIDHNQYKFGHTKVFFKAGLLGLLEEMRDERLSRIITRMQAQ ARGQLMRIEFKKIVERRDALLVIQWNIRAFMGVKNWPWMKLYFKIKPLLKSAE TEKEMATMKEEFGRIKETLEKSEARRKELEEKMVSLLQEKNDLQLQVQAEQD NLNDAEERCDQLIKNKIQLEAKVKEMNERLEDEEEMNAELTAKKRKLEDECS ELKKDIDDLELTLAKVEKEKHATENKVKNLTEEMAGLDEIIAKLTKEKKALQEA HQQALDDLQVEEDKVNSLSKSKVKLEQQVDDLEGSLEQEKKVRMDLERAKR KLEGDLKLTQESIMDLENDKLQLEEKLKKKEFDINQQNSKIEDEQVLALQLQK KLKENQARIEELEEELEAERTARAKVEKLRSDLSRELEEISERLEEAGGATSV QIEMNKKREAEFQKMRRDLEEATLQHEATAAALRKKHADSVAELGEQIDNLQ RVKQKLEKEKSEFKLELDDVTSNMEQIIKAKANLEKVSRTLEDQANEYRVKLE EAQRSLNDFTTQRAKLQTENGELARQLEEKEALISQLTRGKLSYTQQMEDLK RQLEEEGKAKNALAHALQSARHDCDLLREQYEEETEAKAELQRVLSKANSEV AQWRTKYETDAIQRTEELEEAKKKLAQRLQDAEEAVEAVNAKCSSLEKTKHR LQNEIEDLMVDVERSNAAAAALDKKQRNFDKILAEWKQKYEESQSELESSQK EARSLSTELFKLKNAYEESLEHLETFKRENKNLQEEISDLTEQLGEGGKNVHE LEKVRKQLEVEKLELQSALEEAEASLEHEEGKILRAQLEFNQIKAEIERKLAEK DEEMEQAKRNHQRVVDSLQTSLDAETRSRNEVLRVKKKMEGDLNEMEIQLS HANRMAAEAQKQVKSLQSLLKDTQIQLDDAVRANDDLKENIAIVERRNNLLQA ELEELRAVVEQTERSRKLAEQELIETSERVQLLHSQNTSLINQKKKMESDLTQ LQSEVEEAVQECRNAEEKAKKAITDAAMMAEELKKEQDTSAHLERMKKNME QTIKDLQHRLDEAEQIALKGGKKQLQKLEARVRELEGELEAEQKRNAESVKG MRKSERRIKELTYQTEEDKKNLLRLQDLVDKLQLKVKAYKRQAEEAEEQANT NLSKFRKVQHELDEAEERADIAESQVNKLRAKSRDIGAKQKMHDEE
Expression Pattern: Source: Human Protein Atlas Date: 10.11.2017	Selective cytoplasmic expression in heart and skeletal muscle.

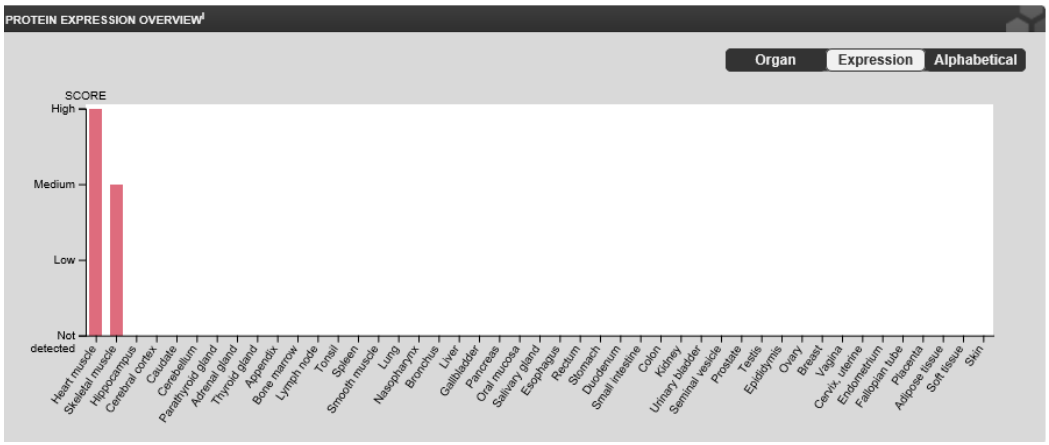

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas: Proteinatlas.org)

Results for Job *Myh6*

Email:	hapke_n@ukw.de
Job Code:	9590327a940d1451451690b9aa14015e-32
Description:	Myh6
Date:	Mon Dec 11 23:32:23 2017

Download the text file of prediction results, suitable for import into spreadsheet software or R

Predicted cleavage sites of multiple protease families

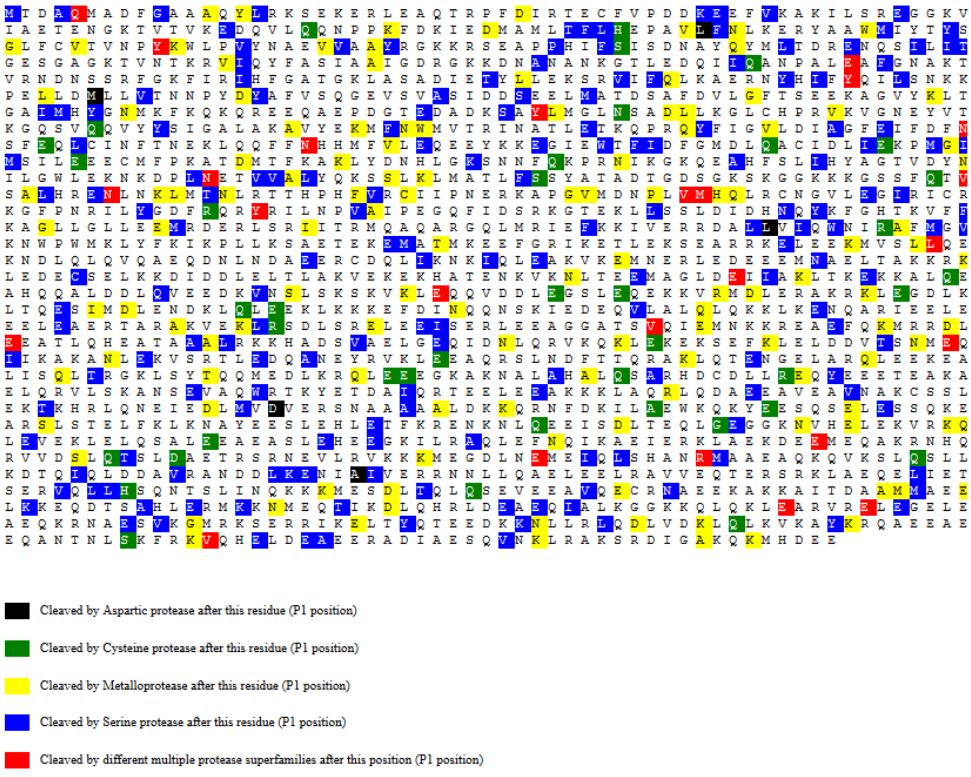

Figure 2: Proteases cleaving sites (Source: Prosper: https://prosper.erc.monash.edu.au/)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=9590327a940d1451451690b9aa14015e-32

Table I: MHC-II prediction (Source: IEDB: http://tools.immuneepitope.org/mhcii/)

	DRB1*01*01

Number of peptides < 10 Percentile Rank	87
Number of Peptide cores with high affinity (< Ic50 50 nM)	87
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	$MYH6_{90-104}$	MAML**TFLHEPAVL**FN	1.62
DRB1*01*01	$MYH6_{125-139}$	VNPY**KWLPVYNAE**VV	6.42
DRB1*01*01	$MYH6_{189-204}$	KRVI**QYFASIAAI**GD	5.11
DRB1*01*01	$MYH6_{264-278}$	IE**TYLLEKSRV**IFQL	3.95
DRB1*01*01	$MYH6_{270-284}$	**EKSRVIFQL**KAERNY	7.88
DRB1*01*01	$MYH6_{272-286}$	S**RVIFQLKAE**RNYHI	6.87
DRB1*01*01	$MYH6_{284-298}$	**YHIFYQILS**NKKPEL	4.16
DRB1*01*01	$MYH6_{285-299}$	HI**FYQILSNKK**PELL	3.95
DRB1*01*01	$MYH6_{347-361}$	KAG**VYKLTGAIM**HYG	5.40
DRB1*01*01	$MYH6_{384-398}$	KS**AYLMGLNSA**DLLK	3.24
DRB1*01*01	$MYH6_{387-401}$	**YLMGLNSAD**LLKGLC	9.63
DRB1*01*01	$MYH6_{394-408}$	ADL**LKGLCHPRV**KVG	9.06

DRB1*01*01	MYH6$_{418-432}$	VQ**QVYYSIGAL**AKAV	2.74
DRB1*01*01	MYH6$_{421-435}$	VY**YSIGALAK**AVYEK	7.88
DRB1*01*01	MYH6$_{429-443}$	AKAV**YEKMFNWMV**TR	5.59
DRB1*01*01	MYH6$_{431-445}$	AVYEK**MFNWMVTRI**N	4.77
DRB1*01*01	*MYH6$_{614-628}$	**SLKLMATLF**SSYATA	3.95
DRB1*01*01	MYH6$_{615-629}$	LKL**MATLFSSYA**TAD	4.16
DRB1*01*01	MYH6$_{643-657}$	GSS**FQTVSALHR**ENL	9.25
DRB1*01*01	MYH6$_{721-735}$	RQR**YRILNPVAI**PEG	0.19
DRB1*01*01	MYH6$_{784-798}$	SRI**ITRMQAQAR**GQL	7.48
DRB1*01*01	MYH6$_{1270-1284}$	RSLN**DFTTQRAKL**QT	8.42
DRB1*01*01	MYH6$_{1565-1579}$	LEFNQ**IKAEIERKL**A	9.45
DRB1*01*01	MYH6$_{1712-1726}$	SERVQ**LLHSQNTSL**I	5.40

* Experimental evidence

Protein name:	Myl2 (Myosin Light Chain 2, Ventricular)
Species:	*Homo sapiens*
Mass/Length	18.8 KDa/ 166 aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MAPKKAKKRAGGANSNVFSMFEQTQIQEFKEAFTIMD QNRDGFIDKNDLRDTFAALGRVNVKNEEIDEMIKEAPG PINFTVFLTMFGEKLKGADPEETILNAFKVFDPEGKGVL KADYVREMLTTQAERFSKEEVDQMFAAFPPDVTGNLD YKNLVHIITHGEEKD
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Highly expressed in the heart muscle; medium expression in Sk muscle. Not expressed in other tissues.

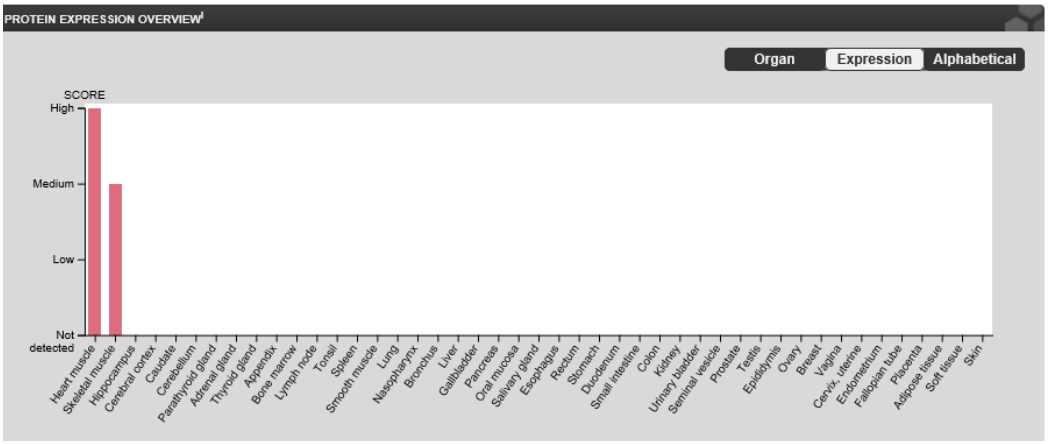

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas)

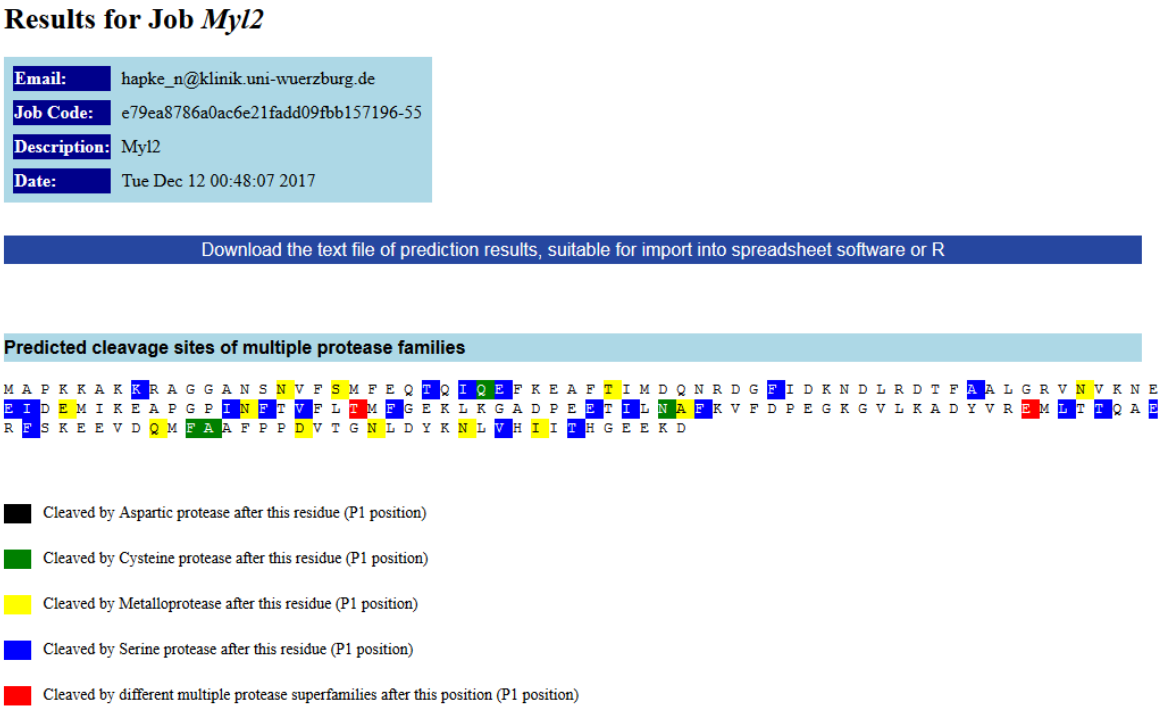

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=e79ea8786a0ac6e21fadd09fbb157196-55

Table I: MHC-II prediction (Source: IEDB)

	HLA DRB1*01*01
Number of peptides < 10 Percentile Rank	7
Number of Peptide cores with high affinity (< Ic50 50 nM)	7
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide core	Peptide position	Percentile Rank
DRB1*01*01	$MYL2_{79-94}$	FTV**FLTMFGEKL**KGA	0.19

Protein name:	Tnni3 (Troponin I, cardiac muscle)
Species:	*Homo sapiens*
Mass/Length	24.008 kDa/210 aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MADGSSDAAREPRPAPAPIRRRSSNYRAYATEPHAKKK SKISASRKLQLKTLLLQIAKQELEREAEERRGEKGRALST RCQPLELAGLGFAELQDLCRQLHARVDKVDEERYDIEAK VTKNITEIADLTQKIFDLRGKFKRPTLRRVRISADAMMQAL LGARAKESLDLRAHLKQVKKEDTEKENREVGDWRKNID ALSGMEGRKKKFES
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Cytoplasmic expression in cardiac myocytes, Very little expression in other tissues.

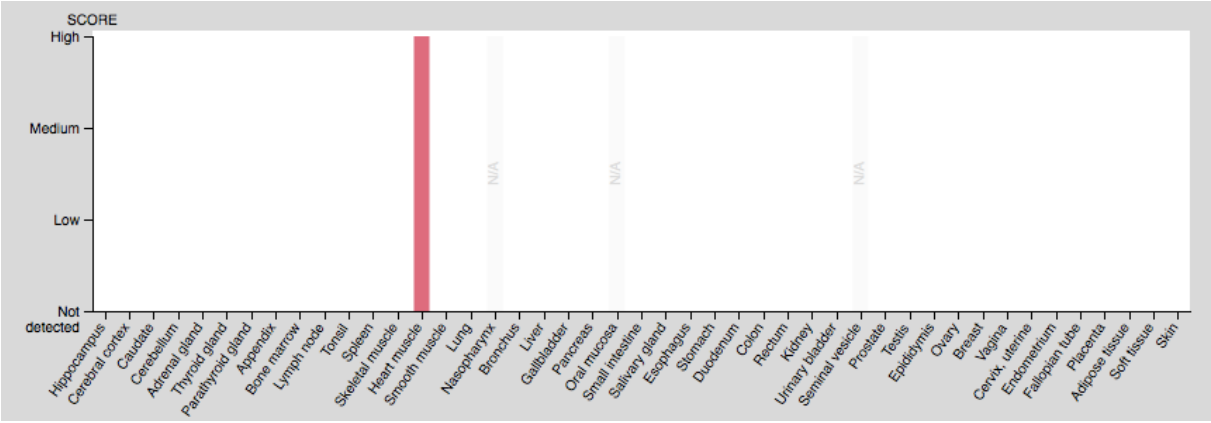

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas)

Results for Job *Tnni3*

Email:	hapke_n@klinik.uni-wuerzburg.de
Job Code:	490237af20af3b817ca70957019318d7-63
Description:	Tnni3
Date:	Tue Dec 12 00:38:17 2017

Download the text file of prediction results, suitable for import into spreadsheet software or R

Predicted cleavage sites of multiple protease families

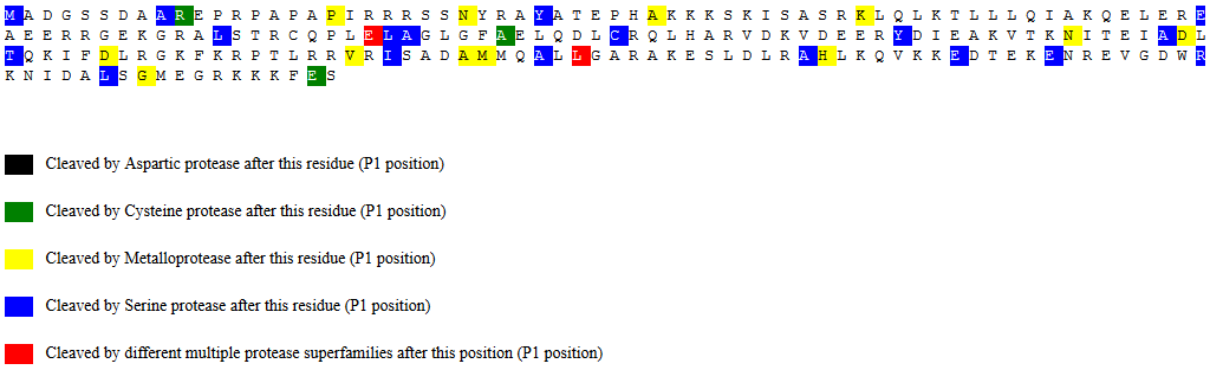

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=490237af20af3b817ca70957019318d7-63

Table I: MHC-II prediction (Source: IEDB)

	DRB1*01*01
Number of peptides < 10 Percentile Rank	13
Number of Peptide cores with high affinity (< Ic50 50 nM)	13
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Haplotype	Peptide core	Peptide position	Percentile
DRB1*01*01	TNNI3$_{43-58}$	ASR**KLQLKTLLL**QIA	7.1
DRB1*01*01	TNNI3$_{149-163}$	ISADAM**MQALLGARA**	3.97
DRB1*01*01	TNNI3$_{152-167}$	DAMM**QALLGARAK**ES	1.15

Protein name:	**Tnnt2(Troponin T Type 2, cardiac)**
Species:	*Homo sapiens*
Mass/Length	35.924 kDa/298 aa
FASTA Sequence : Source: Uniprot Date: 10.11.2017	MSDIEEVVEEYEEEEQEEAAVEEEEDWREDEDEQEEA AEEDAEAEAETEETRAEEDEEEEEAKEAEDGPMEESK PKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNEL QALIEAHFENRKKEEEELVSLKDRIERRRAERAEQQRIR NEREKERQNRLAEERARREEEENRRKAEDEARKKKAL SNMMHFGGYIQKQAQTERKSGKRQTEREKKKKILAER RKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEK FKQQKYEINVLRNRINDNQKVSKTRGKAKVTGRWK
Expression Pattern: Source: Human Protein Atlas Date: 10.11.2017	Selective cytoplasmic expression in cardiac myocytes.

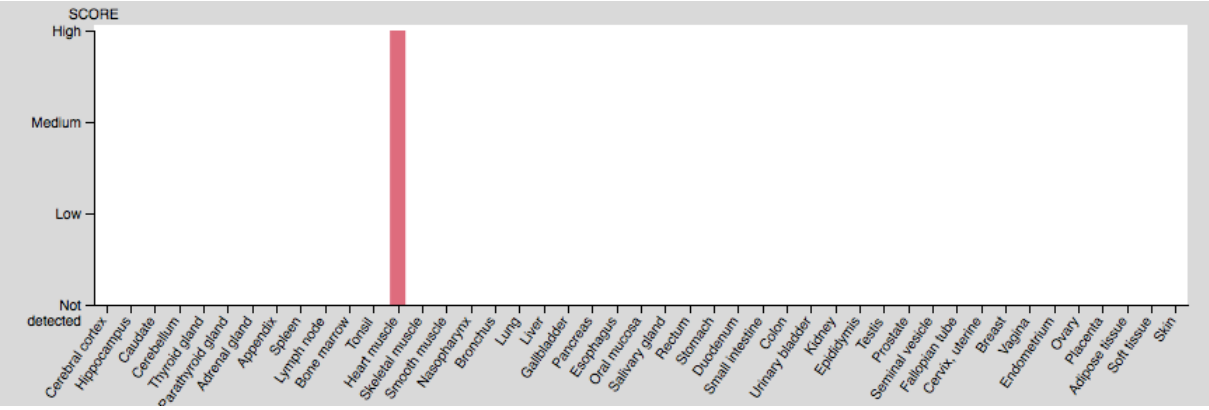

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas)

Results for Job *Tnnt2*

Email:	hapke_n@klinik.uni-wuerzburg.de
Job Code:	24701e969c4b17ef1082732097a28c9e-47
Description:	Tnnt2
Date:	Tue Dec 12 00:37:46 2017

Download the text file of prediction results, suitable for import into spreadsheet software or R

Predicted cleavage sites of multiple protease families

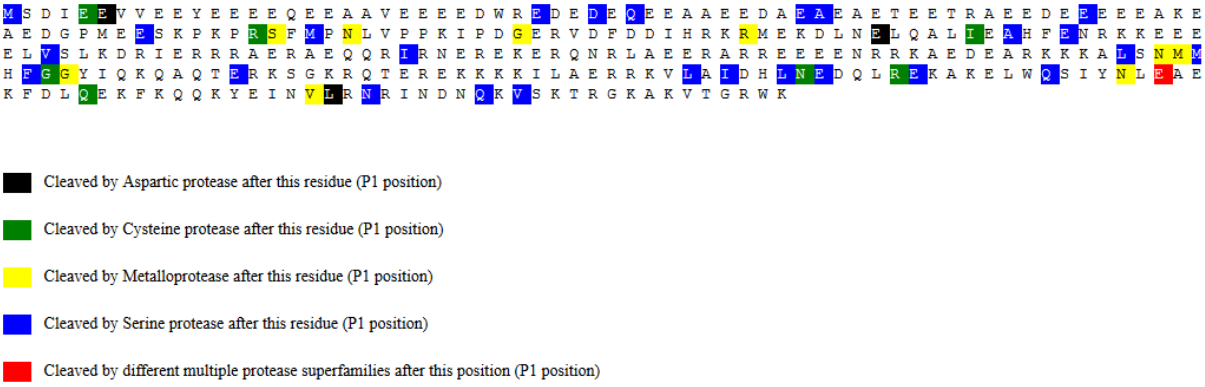

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=24701e969c4b17ef1082732097a28c9e-47

	DRB1*01*01
Number of peptides < 10 Percentile Rank	0
Number of Peptide cores with high affinity (< Ic50 50 nM)	0
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table I: MHC-II prediction (Source: IEDB)

Haplotype	Peptide core	Peptide position	Percentile
DRB1*01*01	$TNNT2_{108-122}$*	LNE**LQALIEAHF**ENR	22.57*

* In silico simulations do not predict DRB1 binding. However, data from DRB1 eluates indicate this peptide can be processed and presented.

Protein name:	**HSPB3 (Heat-shock protein 27)**
Species:	*Homo sapiens*
Mass/Length	16,966 kDa/150 aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MAKIILRHLIEIPVRYQEEFEARGLEDCRLDHALYALPGPT IVDLRKTRAAQSPPVDSAAETPPREGKSHFQILLDVVQFL PEDIIIQTFEGWLLIKAQHGTRMDEHGFISRSFTRQYKLP DGVEIKDLSAVLCHDGILVVEVKDPVGTK
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Heart-enhanced

Pending normal tissue annotation.

RNA:

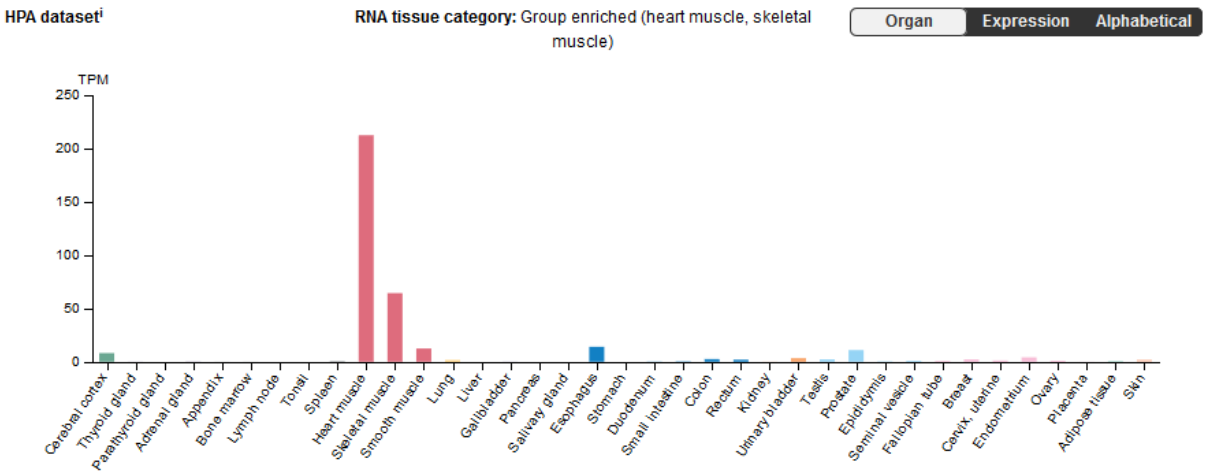

Figure 1: RNA Expression across different tissues (Source Human Protein Atlas)

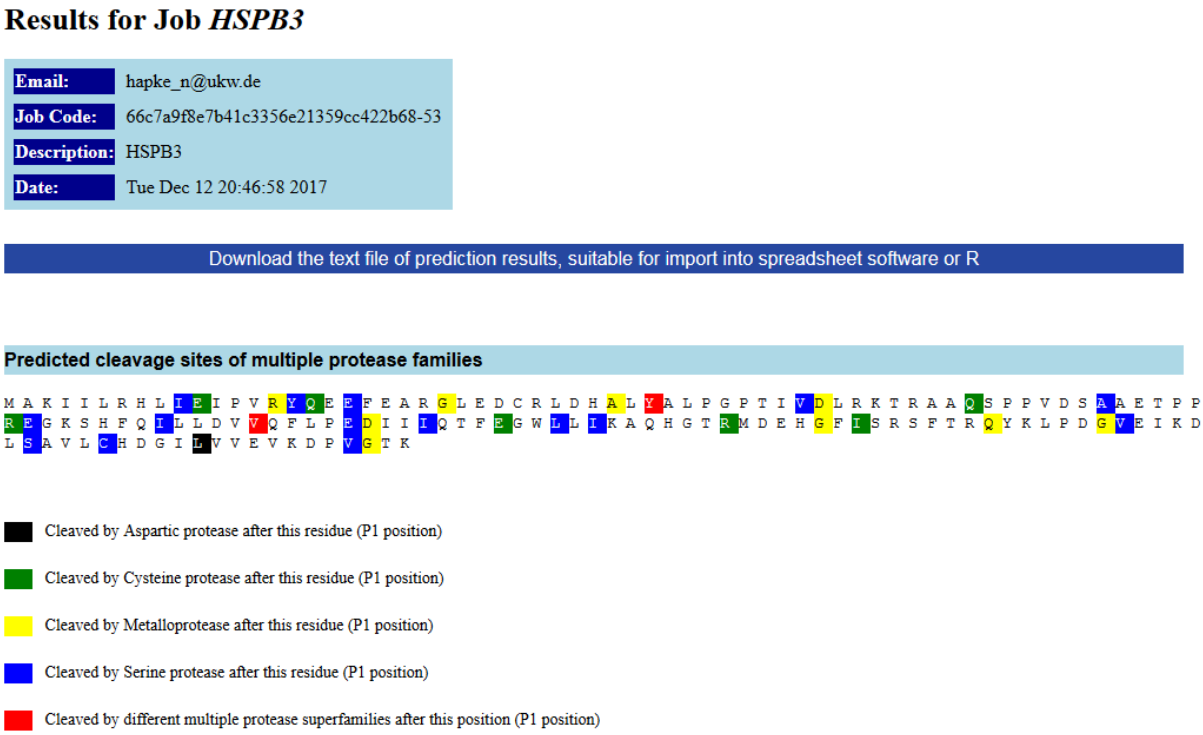

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=66c7a9f8e7b41c3356e21359cc422b68-53

	DRB1*01*01
Number of peptides < 10 Percentile Rank	17
Number of Peptide cores with high affinity (< Ic50 50 nM)	17
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table I: MHC-II prediction (Source: IEDB)

Haplotype	Peptide Position	Peptide Core	Percentile
DRB1*01*01	$HSPB3_{1-15}$	MAKII**LRHLIEIPV**R	9.62
DRB1*01*01	$HSPB3_{31-45}$	DHA**LYALPGPTI**VDL	1.36
DRB1*01*01	$HSPB3_{87-101}$	**IQTFEGWLL**IKAQHG	6.42
DRB1*01*01	$HSPB3_{90-104}$	FE**GWLLIKAQH**GTRM	5.40

Protein name:	**HSPA1A (Heat-Shock Protein 72)**
Species:	*Homo sapiens*
Mass/Length	70,052 kDa/641 aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MAKAAAIGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPS YVAFTDTERLIGDAAKNQVALNPQNTVFDAKRLIGRKFG DPVVQSDMKHWPFQVINDGDKPKVQVSYKGETKAFYPE EISSMVLTKMKEIAEAYLGYPVTNAVITVPAYFNDSQRQA TKDAGVIAGLNVLRIINEPTAAAIAYGLDRTGKGERNVLIF DLGGGTFDVSILTIDDGIFEVKATAGDTHLGGEDFDNRLV NHFVEEFKRKHKKDISQNKRAVRRLRTACERAKRTLSSS TQASLEIDSLFEGIDFYTSITRARFEELCSDLFRSTLEPVE KALRDAKLDKAQIHDLVLVGGSTRIPKVQKLLQDFFNGR DLNKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDV APLSLGLETAGGVMTALIKRNSTIPTKQTQIFTTYSDNQP GVLIQVYEGERAMTKDNNLLGRFELSGIPPAPRGVPQIE VTFDIDANGILNVTATDKSTGKANKITITNDKGRLSKEEIE RMVQEAEKYKAEDEVQRERVSAKNALESYAFNMKSAVE DEGLKGKISEADKKKVLDKCQEVISWLDANTLAEKDEFE HKRKELEQVCNPIISGLYQGAGGPGPGGFGAQGPKGGS GSGPTIEEVD
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Mixed, associated with inflammation, increased expression in the failing heart

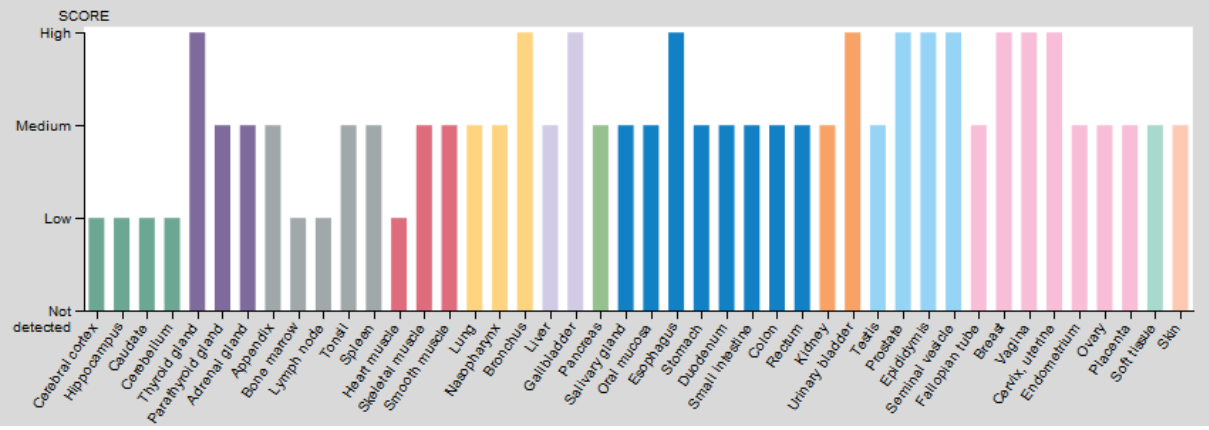

Figure 1: Protein Expression across different tissues (Source Human Protein Atlas)

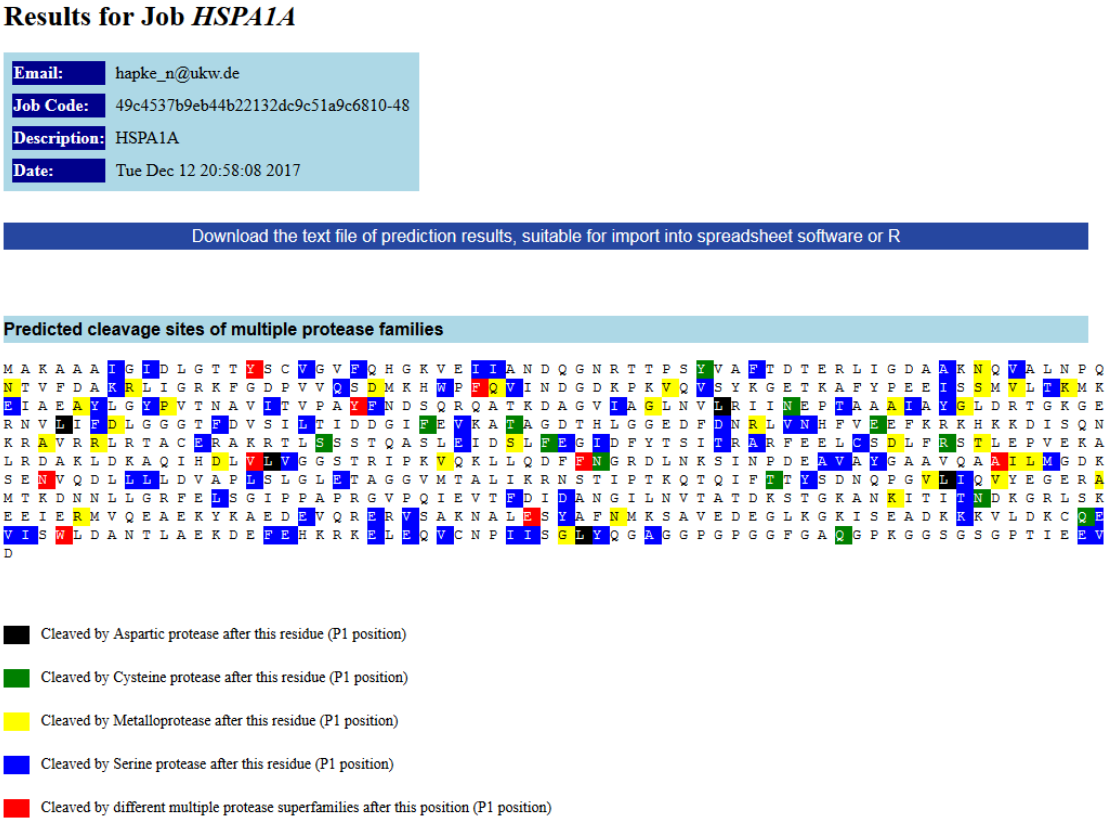

Figure 2: Proteases cleaving sites (Source: Prosper)
https://prosper.erc.monash.edu.au/result3_queue.pl?id=49c4537b9eb44b22132dc9c51a9c6810-48

Table I: MHC-II prediction (Source: IEDB)

	DRB1*01*01
Number of peptides < 10 Percentile Rank	15
Number of Peptide cores with high affinity (< Ic50 50 nM)	15
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Table II: Peptides of interest

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	*HSPA1A$_{168-182}$	NVL**RIINEPTAA**AIAY	*4.05
DRB1*01*01	HSPA1A $_{366-380}$	D**EAVAYGAAV**QAAIL	9.87
DRB1*01*01	HSPA1A $_{388-402}$	VQD**LLLLDVAPL**SLG	4.22

* Experimental evidence

Protein name:	FGA (Fibrinogen alpha chain)
Species:	*Homo sapiens*
Mass/Length	866aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MFSMRIVCLVLSVVGTAWTADSGEGDFLAEGGGVRGPRVVERHQSACKDSD WPFCSDEDWNYKCPSGCRMKGLIDEVNQDFTNRINKLKNSLFEYQKNNKDSH SLTTNIMEILRGDFSSANNRDNTYNRVSEDLRSRIEVLKRKVIEKVQHIQLLQKN VRAQLVDMKRLEVDIDIKIRSCRGSCSRALAREVDLKDYEDQQKQLEQVIAKDL LPSRDRQHLPLIKMKPVPDLVPGNFKSQLQKVPPEWKALTDMPQMRMELERP GGNEITRGGSTSYGTGSETESPRNPSSAGSWNSGSSGPGSTGNRNPGSSGT GGTATWKPGSSGPGSTGSWNSGSSGTGSTGNQNPGSPRPGSTGTWNPGSS ERGSAGHWTSESSVSGSTGQWHSESGSFRPDSPGSGNARPNNPDWGTFEE VSGNVSPGTRREYHTEKLVTSKGDKELRTGKEKVTSGSTTTTRRSCSKTVTKT VIGPDGHKEVTKEVVTSEDGSDCPEAMDLGTLSGIGTLDGFRHRHPDEAAFFD TASTGKTFPGFFSPMLGEFVSETESRGSESGIFTNTKESSSHHPGIAEFPSRGK SSSYSKQFTSSTSYNRGDSTFESKSYKMADEAGSEADHEGTHSTKRGHAKSR PVRDCDDVLQTHPSGTQSGIFNIKLPGSSKIFSVYCDQETSLGGWLLIQQRMDG SLNFNRTWQDYKRGFGSLNDEGEGEFWLGNDYLHLLTQRGSVLRVELEDWA GNEAYAEYHFRVGSEAEGYALQVSSYEGTAGDALIEGSVEEGAEYTSHNNMQ FSTFDRDADQWEENCAEVYGGGWWYNNCQAANLNGIYYPGGSYDPRNNSPY EIENGVVWVSFRGADYSLRAVRMKIRPLVTQ
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Serum, accumulates in the injured heart

	DRB1*01*01
Number of peptides < 10 Percentile Rank	20
Number of Peptide cores with high affinity (< Ic50 50 nM)	20
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	§*FGA$_{79-93}$	Q**DFTNRINK**LKNSLFE	8.84
DRB1*01*01	§*FGA$_{81-95}$	**FTNRINKL**KNSLFEY	6.87
DRB1*01*01	FGA $_{83-97}$	NRINKL**KNSLFEYQK**	7.08
DRB1*01*01	FGA $_{528-542}$	T**FPGFFSPML**GEFVS	4.64
DRB1*01*01	FGA $_{530-544}$	PGF**FSPMLGEFV**SET	3.24
DRB1*01*01	FGA$_{536-550*}$	GF**FSPMLGEFV**SETE	3.80

* Experimental evidence

§ Citrulination reported

Protein name:	**FGB (Fibrinogen beta chain)**
Species:	*Homo sapiens*
Mass/Length	55,92 KDa, 491aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MKRMVSWSFHKLKTMKHLLLLLLCVFLVKSQGVNDNEEGFFSARGHRPLDKK REEAPSLRPAPPPISGGGYRARPAKAAATQKKVERKAPDAGGCLHADPDLGVL CPTGCQLQEALLQQERPIRNSVDELNNNVEAVSQTSSSSFQYMYLLKDLWQKR QKQVKDNENVVNEYSSELEKHQLYIDETVNSNIPTNLRVLRSILENLRSKIQKLE SDVSAQMEYCRTPCTVSCNIPVVSGKECEEIIRKGGETSEMYLIQPDSSVKPYR VYCDMNTENGGWTVIQNRQDGSVDFGRKWDPYKQGFGNVATNTDGKNYCGL PGEYWLGNDKISQLTRMGPTELLIEMEDWKGDKVKAHYGGFTVQNEANKYQIS VNKYRGTAGNALMDGASQLMGENRTMTIHNGMFFSTYDRDNDGWLTSDPRK QCSKEDGGGWWYNRCHAANPNGRYYWGGQYTWDMAKHGTDDGVVWMNW KGSWYSMRKMSMKIRPFFPQQ
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Serum, accumulates in the injured heart

	DRB1*01*01
Number of peptides < 10 Percentile Rank	33
Number of Peptide cores with high affinity (< Ic50 50 nM)	33
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	FGB_{67-81}	SGGG**YRARPAKA**AAT	9.63
DRB1*01*01	$FGB_{252-266}$	SEM**YLIQPDSSV**KPY	8.47
DRB1*01*01	$FGB_{372-386}$	VNK**YRGTAGNA**LMDG	4.72
DRB1*01*01	$FGB_{328-342}$	KIS**QLTRMGPTE**LLI	6.87

Protein name:	FGG (Fibrinogen gamma chain)
Species:	*Homo sapiens*
Mass/Length	453aa
FASTA Sequence : Source: Uniprot Date: 17.10.2017	MSWSLHPRNLILYFYALLFLSSTCVAYVATRDNCCILDERFGSYCPTTCGIADFL STYQTKVDKDLQSLEDILHQVENKTSEVKQLIKAIQLTYNPDESSKPNMIDAATL KSRKMLEEIMKYEASILTHDSSIRYLQEIYNSNNQKIVNLKEKVAQLEAQCQEPC KDTVQIHDITGKDCQDIANKGAKQSGLYFIKPLKANQQFLVYCEIDGSGNGWTV FQKRLDGSVDFKKNWIQYKEGFGHLSPTGTTEFWLGNEKIHLISTQSAIPYALR VELEDWNGRTSTADYAMFKVGPEADKYRLTYAYFAGGDAGDAFDGFDFGDDP SDKFFTSHNGMQFSTWDNDNDKFEGNCAEQDGSGWWMNKCHAGHLNGVYY QGGTYSKASTPNGYDNGIIWATWKTRWYSMKKTTMKIIPFNRLTIGEGQQHHL GGAKQVRPEHPAETEYDSLYPEDDL
Expression Pattern: Source: Human Protein Atlas Date: 17.10.2017	Serum, accumulates in the injured heart

	DRB1*01*01
Number of peptides < 10 Percentile Rank	35
Number of Peptide cores with high affinity (< Ic50 50 nM)	35
Number of Peptide cores with mid-affinity (< Ic50 500 nM)	0
Number of Peptide cores with low-affinity (< Ic50 5000 nM + < Percentile 10)	0

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	FGG$_{189-203}$	Q**SGLYFIKPL**KANQQ	5.59
DRB1*01*01	FGG $_{238-252}$	KEG**FGHLSPTGT**TEF	3.49
DRB1*01*01	FGG $_{256-270}$	NE**KIHLISTQS**AIPY	7.88
DRB1*01*01	FGG $_{296-310}$	EAD**KYRLTYA**YFAGG	4.77

Previously described Citrulinated epitoes

Haplotype	Peptide Position	Peptide core	Percentile
DRB1*01*01	*FGA$_{79-93}$	Q**DFTNRINK**LKNSLFE (+ CIT R6)	9.04
DRB1*01*01	FGA$_{81-95}$	**FTNRINKL**KNSLFEY (+ CIT R4)	7.28

Guideline to Peptide Identification

1-01-001

Species

0 Mouse

1 Human

Protein Id

01 ACTC1

02 ADRB1

03 MYBPC3

04 MYH6

05 MYL2

06 TNNI3

07 TNNT2

08 HSPB3

09 HSPA1A

10 FGA

11 FGB

12 FGG

13 CIT

Peptide cumulative numerical order

For mouse: **1-193**

For Human: **1-95**

Thus, the above-mentioned example refers to a peptide derived from the human (1) ACTC1 protein (01), and it is the first peptide from that library (001).

Peptide Library of Human, DRB1-restricted cardiac antigens

Haplotype	$Protein_{peptide}$	Sequence	%	Id
ACTC1				
DRB1*01*01	ACTC1$_{132-146}$	PA**MYVAIQAVL**SLYA	0.76	1-01-001
DRB1*01*01	ACTC1$_{137-150}$	AIQA**VLSLYASGR**TT	7.08	1-01-002
DRB1*01*01	ACTC1$_{265-279}$	QPS**FIGMESAG**IHET	0.97	1-01-003
DRB1*01*01	ACTC1$_{316-330}$	QKE**ITALAPST**MKIK	4.16	1-01-004
ADRB1				
DRB1*01*01	ADRB1$_{28-44}$	TA**ARLLVPASP**PASLLP	5.46	1-02-005
DRB1*01*01	ADRB1$_{63-77}$	**LLMALIVLL**IVAGNV	9.01	1-02-006
DRB1*01*01	ADRB1$_{67-81}$	LIV**LLIVAGNVL**VIV	1.36	1-02-007
DRB1*01*01	ADRB1$_{75-89}$	**GNVLVIVAI**AKTPRL	8.28	1-02-008
DRB1*01*01	ADRB1$_{76-90}$	NV**LVIVAIAKT**PRLQ	9.87	1-02-009
DRB1*01*01	ADRB1$_{92-106}$	**LTNLFIMSL**ASADLV	0.88	1-02-010
DRB1*01*01	ADRB1$_{93-107}$	TN**LFIMSLASA**DLVM	0.77	1-02-011
DRB1*01*01	ADRB1$_{96-110}$	F**IMSLASADL**VMGLL	3.24	1-02-012
DRB1*01*01	ADRB1$_{97-111}$	IMSLAS**ADLVMGLLV**	5.59	1-02-013
DRB1*01*01	*ADRB1$_{107-121}$	MGL**LVVPFGATI**VVW	7.51	1-02-014
DRB1*01*01	ADRB1$_{153-167}$	ALD**RYLAITSPF**RYQ	6.65	1-02-015
DRB1*01*01	ADRB1$_{164-178}$	FR**YQSLLTRAR**ARGL	4.95	1-02-016
DRB1*01*01	ADRB1$_{167-181}$	**QSLLTRARA**RGLVCT	7.48	1-02-017
DRB1*01*01	ADRB1$_{168-182}$	SL**LTRARARGL**VCTV	8.87	1-02-018
DRB1*01*01	ADRB1$_{177-191}$	GLV**CTVWAISAL**VSF	8.67	1-02-019
DRB1*01*01	ADRB1$_{215-229}$	CCD**FVTNRAYAI**ASS	0.62	1-02-020
DRB1*01*01	ADRB1$_{220-234}$	**TNRAYAIAS**SVVSFY	3.24	1-02-021
DRB1*01*01	ADRB1$_{221-235}$	NRAYAI**ASSVVSFYV**	3.02	1-02-022
DRB1*01*01	ADRB1$_{311-325}$	**PSRLVALRE**QKALKT	6.00	1-02-023
DRB1*01*01	ADRB1$_{312-326}$	SRL**VALREQKAL**KTL	5.59	1-02-024
DRB1*01*01	ADRB1$_{334-348}$	TLCWLP**FFLANVVKA**	8.67	1-02-025
DRB1*01*01	ADRB1$_{335-349}$	LCWLPF**FLANVVKAF**	6.52	1-02-026
MYBPC3				
DRB1*01*01	MYBPC3$_{76-90}$	QG**SYAVIAGSS**KVKF	6.87	1-03-027
DRB1*01*01	MYBPC3$_{171-185}$	SIT**FSARVAGAS**LLK	6.46	1-03-028
DRB1*01*01	MYBPC3$_{346-360}$	RGM**LKRLKGMRR**DEK	3.95	1-03-029
DRB1*01*01	MYBPC3$_{409-423}$	**KYIFESIGA**KRTLTI	1.15	1-03-030
DRB1*01*01	MYBPC3$_{410-424}$	Y**IFESIGAKR**TLTIS	1.36	1-03-031
DRB1*01*01	MYBPC3$_{412-426}$	FESIGA**KRTLTISQC**	5.79	1-03-032
DRB1*01*01	MYBPC3$_{444-458}$	STE**LFVKEPPVL**ITR	2.27	1-03-033
DRB1*01*01	MYBPC3$_{544-558}$	**KLEVYQSIA**DLMVGA	5.95	1-03-034
DRB1*01*01	MYBPC3$_{545-559}$	LEVYQS**IADLMVGAK**	5.43	1-03-035
DRB1*01*01	MYBPC3$_{618-632}$	PEG**FACNLSAKL**HFM	7.88	1-03-036
DRB1*01*01	MYBPC3$_{656-670}$	PDT**IVVVAGNKL**RLD	3.71	1-03-037
DRB1*01*01	MYBPC3$_{936-950}$	TG**ARLLFRVRA**HNMA	9.06	1-03-038
DRB1*01*01	MYBPC3$_{1023-1037}$	NSPT**DTILFIRAA**RR	7.08	1-03-039
DRB1*01*01	MYBPC3$_{1026-1040}$	TDTI**LFIRAARRV**HS	4.72	1-03-040
DRB1*01*01	MYBPC3$_{1134-1148}$	GYY**FRVFSQNMV**GFS	5.59	1-03-041
DRB1*01*01	MYBPC3$_{1224-1238}$	DARFRM**FSKQGVLTL**	8.09	1-03-042

MYH6				
DRB1*01*01	MYH6$_{90-104}$	MAML**TFLHEPAVL**FN	1.62	1-04-043
DRB1*01*01	MYH6$_{125-139}$	VNPY**KWLPVYNAE**VV	6.42	1-04-044
DRB1*01*01	MYH6$_{189-204}$	KRVI**QYFASIAAI**GD	5.11	1-04-045
DRB1*01*01	MYH6$_{264-278}$	IE**TYLLEKSRV**IFQL	3.95	1-04-046
DRB1*01*01	MYH6$_{270-284}$	**EKSRVIFQL**KAERNY	7.88	1-04-047
DRB1*01*01	MYH6$_{272-286}$	S**RVIFQLKAE**RNYHI	6.87	1-04-048
DRB1*01*01	MYH6$_{284-298}$	**YHIFYQILS**NKKPEL	4.16	1-04-049
DRB1*01*01	MYH6$_{285-299}$	HI**FYQILSNKK**PELL	3.95	1-04-050
DRB1*01*01	MYH6$_{347-361}$	KAG**VYKLTGAIM**HYG	5.40	1-04-051
DRB1*01*01	MYH6$_{384-398}$	KS**AYLMGLNSA**DLLK	3.24	1-04-052
DRB1*01*01	MYH6$_{387-401}$	**YLMGLNSAD**LLKGLC	9.63	1-04-053
DRB1*01*01	MYH6$_{394-408}$	ADL**LKGLCHPRV**KVG	9.06	1-04-054
DRB1*01*01	MYH6$_{418-432}$	VQ**QVYYSIGAL**AKAV	2.74	1-04-055
DRB1*01*01	MYH6$_{421-435}$	VY**YSIGALAK**AVYEK	7.88	1-04-056
DRB1*01*01	MYH6$_{429-443}$	AKAV**YEKMFNWMV**TR	5.59	1-04-057
DRB1*01*01	MYH6$_{431-445}$	AVYEK**MFNWMVTRI**N	4.77	1-04-058
DRB1*01*01	*MYH6$_{614-628}$	**SLKLMATLF**SSYATA	3.95	1-04-059
DRB1*01*01	MYH6$_{615-629}$	LKL**MATLFSSYA**TAD	4.16	1-04-060
DRB1*01*01	MYH6$_{643-657}$	GSS**FQTVSALHR**ENL	9.25	1-04-061
DRB1*01*01	MYH6$_{721-735}$	RQR**YRILNPVAI**PEG	0.19	1-04-062
DRB1*01*01	MYH6$_{784-798}$	SRI**ITRMQAQAR**GQL	7.48	1-04-063
DRB1*01*01	MYH6$_{1270-1284}$	RSLN**DFTTQRAKL**QT	8.42	1-04-064
DRB1*01*01	MYH6$_{1565-1579}$	LEFNQ**IKAEIERKL**A	9.45	1-04-065
DRB1*01*01	MYH6$_{1712-1726}$	SERVQ**LLHSQNTSL**I	5.40	1-04-066
MYL2				
DRB1*01*01	MYL2$_{79-94}$	FTV**FLTMFGEKL**KGA	0.19	1-05-067
TNNI3				
DRB1*01*01	TNNI3$_{43-58}$	ASR**KLQLKTLLL**QIA	7.1	1-06-068
DRB1*01*01	TNNI3$_{149-163}$	ISADAM**MQALLGARA**	3.97	1-06-069
DRB1*01*01	TNNI3$_{152-167}$	DAMM**QALLGARAK**ES	1.15	1-06-070
TNNT2				
DRB1*01*01	TNNT2$_{108-122}$*	LNE**LQALIEAHF**ENR	22.57*	1-07-071
HSPB3				
DRB1*01*01	HSPB3$_{1-15}$	MAKII**LRHLIEIPV**R	9.62	1-08-072
DRB1*01*01	HSPB3$_{31-45}$	DHA**LYALPGPTI**VDL	1.36	1-08-073
DRB1*01*01	HSPB3$_{87-101}$	**IQTFEGWLL**IKAQHG	6.42	1-08-074
DRB1*01*01	HSPB3$_{90-104}$	FE**GWLLIKAQH**GTRM	5.40	1-08-075
HSPA1A				
DRB1*01*01	*HSPA1A$_{168-182}$	NVL**RIINEPTAA**AIAY	*4.05	1-09-076
DRB1*01*01	HSPA1A $_{366-380}$	D**EAVAYGAAV**QAAIL	9.87	1-09-077
DRB1*01*01	HSPA1A $_{388-402}$	VQD**LLLLDVAPL**SLG	4.22	1-09-078
FGA				
DRB1*01*01	§*FGA$_{79-93}$	Q**DFTNRINK**LKNSLFE	8.84	1-10-079
DRB1*01*01	§*FGA$_{81-95}$	**FTNRINKL**KNSLFEY	9.01	1-10-080

DRB1*01*01	FGA $_{83-97}$	NRINKL**KNSLFEYQK**	6.87	1-10-081
DRB1*01*01	FGA $_{528-542}$	T**FPGFFSPML**GEFVS	7.08	1-10-082
DRB1*01*01	FGA $_{530-544}$	PGF**FSPMLGEFV**SET	4.64	1-10-083
DRB1*01*01	FGA $_{530-544}$	PGF**FSPMLGEFV**SET	3.24	1-10-084
DRB1*01*01	FGA$_{536-550*}$	GF**FSPMLGEFV**SETE	3.80	1-10-085
FGB				
DRB1*01*01	FGB$_{67-81}$	SGGG**YRARPAKA**AAT	9.63	1-11-086
DRB1*01*01	FGB$_{252-266}$	SEM**YLIQPDSSV**KPY	8.47	1-11-087
DRB1*01*01	FGB $_{372-386}$	VNK**YRGTAGNA**LMDG	4.72	1-11-088
DRB1*01*01	FGB $_{328-342}$	KIS**QLTRMGPTE**LLI	6.87	1-11-089
FGG				
DRB1*01*01	FGG$_{189-203}$	Q**SGLYFIKPL**KANQQ	5.59	1-12-090
DRB1*01*01	FGG $_{238-252}$	KEG**FGHLSPTGT**TEF	3.49	1-12-091
DRB1*01*01	FGG $_{256-270}$	NE**KIHLISTQS**AIPY	7.88	1-12-92
DRB1*01*01	FGG $_{296-310}$	EAD**KYRLTYA**YFAGG	4.77	1-12-93
CIT FGA				
DRB1*01*01	*FGA$_{79-93}$	Q**DFTNRINK**LKNSLFE (+ CIT R6)	9.04	1-13-94
DRB1*01*01	FGA$_{81-95}$	**FTNRINKL**KNSLFEY (+ CIT R4)	7.28	1-13-95