

From THE DEPARTMENT OF MEDICAL BIOCHEMISTRY AND
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**AUTOANTIBODY RECOGNITION OF COLLAGEN TYPE II
IN ARTHRITIS**

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ABSTRACT

Autoantibodies against collagen type II (CII), a protein localized in the joint cartilage, play a major role in collagen-induced arthritis (CIA), one of the most commonly used animal models for rheumatoid arthritis (RA). The studies included in this thesis were undertaken to elucidate structural and functional requirements for B and T cells to recognize native CII structures during experimental arthritis as well as in human RA. To reveal in detail how CII-specific autoantibodies recognize CII, we determined the crystal structure of two pathogenic immune complexes occurring in arthritis. The two crystal structures reveal how autoantibodies target triple-helical CII and specifically which epitope sequences and CDR residues that are crucial for binding. Interestingly, although amino acid residues in the hypervariable regions of both autoantibodies were somatically mutated, the majority of the contacts with the epitope involve germline-encoded structures. A recombinant CII peptide library was also generated in order to reveal the CII epitope specificity of polyclonal autoantibodies in CIA. This library was used to confirm already known CII epitopes occurring in CIA but also to investigate new CII epitope regions that could play a role in arthritis. Several new regions targeted by autoantibodies were found and interestingly, antibodies to these regions were also identified in non-human primate species with arthritis. Subsequently, autoantibodies to the major CII epitopes C1, U1, and J1 were analyzed in serum and synovial fluid from RA patients. The autoantibody levels to all three CII epitopes were significantly higher in synovial fluid compared to serum, in particular autoantibodies to the U1 epitope. Finally, we studied the CII-specific antibody responses as well as CII-specific T cell responses during different timepoints of CIA in two mouse strains, B10.Q and a humanized mouse strain B10.DR4.*Ncf1*^{*/*}. The obtained data showed that the antibody and T cell responses investigated stayed relatively constant during the disease course and were dominated by T cells specific for non-modified (K264) and hydroxylated (HOK264) CII259-273 peptides in the humanized mice and to the galactosylated (GalOK264) CII259-273 peptide in the B10.Q mice. C1 specific antibodies dominated in both strains while antibodies to the U1 epitope was characteristic for the humanized mouse.

LIST OF PUBLICATIONS

I. Analysis of autoantibodies to the joint-specific protein collagen II in experimental and rheumatoid arthritis

Ingrid Lindh, Hüseyin Uysal, Omri Snir, Erik Lönnblom, Ida Andersson, Franziska Lange, Michel Vierboom, Kutty Selva Nandakumar, Vivianne Malmström, and Rikard Holmdahl

Manuscript

II. Crystal structure of an arthritogenic anticollagen immune complex

Doreen Dobritzsch*, **Ingrid Lindh***, Hüseyin Uysal, Kutty Selva Nandakumar, Harald Burkhardt, Gunter Schneider, and Rikard Holmdahl

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III. Comparative analysis of CII-specific immune responses during development of collagen induced arthritis in two B10 strains

Tsvetelina Batsalova*, **Ingrid Lindh***, Johan Bäcklund, Balik Dzhambazov, and Rikard Holmdahl

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IV. Immunoglobulin heavy chain V gene polymorphism controls epitope-specific antibody response

Bruno Raposo, Doreen Dobritzsch, **Ingrid Lindh**, Diana Ekman, Changrong Ge, Michael Förster, Hüseyin Uysal, Gunter Schneider, and Rikard Holmdahl

Manuscript

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Tissue transglutaminase enhances collagen type II-induced arthritis and modifies the immunodominant T-cell epitope CII260-270

Balik Dzhambazov, **Ingrid Lindh**, Åke Engström, and Rikard Holmdahl

Eur J Immunol. 2009 Sep;39(9):2412-23.

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LIST OF ABBREVIATIONS

Aa	Amino acid (s)
Ab(s)	Antibody (antibodies)
ACPA	Anti-citrullinated protein antibody
BCR	B-cell receptor
C	Constant
CII	Type II collagen
CB	Cyanogen bromide
CAIA	Collagen antibody induced arthritis
CDR	Complementary determining region
CIA	Collagen induced arthritis
COMP	Cartilage oligomeric matrix protein
D	Diversity
Fab	Fragment antigen binding
Fc	Fragment crystallizable
HLA	Human leukocyte antigen
IFN	Interferon
Ig(s)	Immunoglobulin(s)
IL	Interleukin
J	Joining
MHC	Major histocompatibility complex
pg	Proteoglycan
RA	Rheumatoid arthritis
RF	Rheumatoid factor
TCR	T cell receptor
Th	T helper cell
TNF	Tumor necrosis factor

1 INTRODUCTION TO THE STUDY

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by persistent synovitis, systemic inflammation, and autoantibodies. The disease etiology, symptoms, and pathogenesis are complex and multifaceted. This can be demonstrated by the effectiveness of certain biological therapies in some patients but a complete lack of response in a large number of other patients (1). Discovering subtypes of RA patients is therefore today considered a central research area for the improvement of response to therapy (2,3). Several subtypes of RA patients have been identified based on particular clinical and molecular characteristics (4,5). The studies presented in this thesis are limited to a subgroup of RA patients developing an autoimmune response to the cartilage-specific protein collagen type II (CII) but can be extended to immune responses against other autoantigens as well.

1.1 BACKGROUND

RA is a complex disorder that if left untreated, severely affects cartilage and bone. CII-specific cells from the immune system (B and T cells) as well as autoantibodies and cytokines they secrete can be detected in these local areas as well as in serum (6–10) and several lines of evidence confirm their contribution to the abnormal pathogenic events operating in and around the RA joints (6,11–16). The basic mechanisms occurring in such complex disorders can be studied in controlled animal model systems that provide insights into one or another particular aspect or phase of the “entire” disease. One of the models frequently used is collagen-induced arthritis (CIA). This model share immunological and pathological features with RA (17–20) but there are also differences between the two as CIA is a time-dependent model induced with CII. The latter makes it a well suitable model for studies aiming at understanding CII-specific autoimmunity.

CIA is characterized by high levels of circulating autoantibodies against triple-helical CII, but the specific epitope location and sequences of the antibody binding sites have yet not been fully characterized. This is mainly due to 1) lack of screening systems that cover all possible epitope regions and 2) that studies are limited to the specificity of monoclonal antibodies established very early post immunization. Anti-CII antibodies are pathogenic in mice (18), and except for their characteristic effector functions such as binding and activation of complement, and the activation of FcγR-bearing cells, some of them can also disrupt articular cartilage without any mediators of inflammation (21). The way cartilage and CII is affected depends on the autoantibody specificity (15,16,21,22), suggesting that epitope locations could play a part in these processes. This is also relevant to RA where these types of autoantibodies occur. However, it might be more difficult to identify pathogenic effects in RA compared to CIA since RA is more complex with other pathogenic events that potentially cover the effects of the CII response.

To fully dissect the CII epitope specific response occurring during both CIA and RA, earlier findings need to be complemented. In addition, although previous studies suggest that a consensus motif (R-G-hydrophobic) present in several of the known CII

epitopes, could be a recognition sequence for autoreactive B cells (23), its relevance in CIA and RA has not yet been confirmed.

Not only B cells but also T cells play a critical role in CIA (24–27). The presence of activated CD4⁺ T cells as well as the correlation between RA and the expression of certain MHC class II alleles (in particular certain subtypes of HLA-DR4) (28,29) has raised the possibility that RA is caused by a T cell mediated immune response towards one or a few joint-specific antigens. Both human DR4 and the CIA susceptible mouse expressing H-2^q molecules can present CII to autoreactive T cells (30). The immunodominant T cell epitope on CII (CII259-273) can be post-translationally modified on the lysine (K) residues leading to non-modified, hydroxylated or glycosylated epitopes. In general, the T cell response to the glycosylated T cell epitope dominates in RA and in H-2^q restricted CIA (6,31) whereas the non-modified epitope dominates in the DR4 transgenic mouse (32). Although specific B and T cell responses can be measured at a certain disease timepoint those might not give the complete picture of how CII specific B and T cell responses occur over time nor their relevance to the disease. Thus, studies focusing on CII specific responses over a longer period of time could reveal how B and T cell responses relate to different disease phases and to each other.

1.2 PURPOSE OF THE STUDY

This thesis was undertaken in order to further dissect autoimmunity to CII in CIA and RA. The main purposes as well as relevant research questions are outlined below.

The main purposes of the study were to:

- I. Validate and build a more comprehensive understanding of earlier discoveries of biologically significant CII epitopes in CIA
- II. Investigate the anti-CII antibody specificities in different species and mouse strains with arthritis in order to reveal that this model is relevant and matches the events operating in the human disease.
- III. Perform long-time studies of the CII epitope specific response in order to reveal if epitopes could be categorized into different phases.
- IV. Determine how pathogenic autoantibodies recognize triple-helical CII in order to elucidate how specificities and diversities operate in such immune complexes.
- V. Investigate the magnitude and specificity of the CII specific B and T cell responses over time in different mouse strains and investigate the dissimilarities between sick and healthy mice.

Relevant research questions:

- 1) Could a more comprehensive CII epitope screening library be generated to complement earlier findings?
- 2) Could such a system be useful to screen for all potential CII epitopes detected during different disease phases and help us to understand whether some CII regions are more exposed and susceptible for antibodies to bind?

- 3) Could such a system also be used to investigate CII epitope specificities in different species?
- 4) Could we get a more structured overview of recognition motifs on CII and reveal the existence of early and late phase epitopes?
- 5) Can the CII specific antibody responses vary in different compartments in the body? If so, is that relevant to the disease pathogenesis?
- 6) Could we crystallize anti-CII antibodies together with triple helical CII peptides?
- 7) Could such crystal structures clearly describe the crucial immune complex interactions?
- 8) Do we see the same magnitude and specificity to the native and modified variants of the T cell epitope over time in CIA compared to RA? Will the response differ between mouse strains?

2 INTRODUCTION TO THE IMMUNE SYSTEM

2.1 INNATE AND ADAPTIVE IMMUNITY

If your body is affected by an infection (could be virus, bacteria, fungi, protozoa, etc.) the components of **the innate immune system** will recognize structures on the invading pathogens so-called pathogen associated molecular patterns (**PAMPs**). This system also recognize endogenous (self) molecules created upon tissue injury called damage-associated molecular patterns (**DAMPs**) (33–35). These structures are recognized by pattern recognition receptors (**PRRs**) of which the family of Toll-like receptors (**TLRs**) has been studied most extensively (36,37). The intracellular signaling cascades triggered by these PRRs lead to expression of inflammatory mediators that coordinate the elimination of pathogens and infected cells. In some cases processes that control the inflammatory response work inefficiently, which can lead to immunodeficiencies and induction of **autoimmunity** (*i.e.* an immune response against the body's own cells and/or tissues). The primary cells of the innate immune system are concentrated at epithelial surfaces including the skin, respiratory tract, and gastrointestinal tract. **Dendritic cells** (DCs) are most important because of their ability to take up and process foreign material, then present antigens for a primary immune response (38–41). Further cells in the innate immune system are macrophages, neutrophils, natural killer (NK) cells, basophils, mast cells and eosinophils. Also $\gamma\delta$ -T cells (42) and NK T cells (43) might be considered part of the innate immune system. Not only cells but also the **complement system**, comprising plasma and membrane proteins plays a key role in innate and adaptive response (44,45) where they have an important role to eliminate pathogens and apoptotic cells. Contribution of complement to the development of RA have been described (46), as well as to the development of CIA in mice and other similar rodent models (47–56).

The adaptive immunity provides additional protection due to its capability to respond more specifically and diverse. The molecules responsible for this specific and diverse recognition of antigens are **antibodies** expressed and secreted by **B cells**, and **T cell antigen receptors** (TCRs) expressed on **T cells**. The process describing how antibody specificity and diversity operate will be discussed further in the sections below as well as in study II and IV. Adaptive immunity can be divided into **humoral immunity** and **cell-mediated immunity**. Secreted antibodies are the effector molecules of humoral immunity, capable of neutralizing and eliminating extracellular pathogens by activating various effector mechanisms. The cell-mediated immunity becomes active when pathogens survive inside cells and T cells mediate this type of immunity.

2.2 B AND T CELL DEVELOPMENT

B cells (bursa or bone marrow-derived) express **B cell antigen receptors** (BCRs), which recognize a wide variety of macromolecules and small chemicals. Their origin can be traced back to the evolution of adaptive immunity in jawed vertebrates more than 500 million years ago (57). **T cells** (thymus-derived) are a population of cells expressing **T cell receptors** (TCRs) recognizing small peptides loaded onto **major histocompatibility complexes** (MHCs) molecules.

B and T cells originate from multipotent hematopoietic stem cells in the bone marrow and go through a well-controlled development and educational process before they become fully mature cells ready to be activated and perform their effector functions.

After exiting the bone marrow progenitor T cells enter the thymus as TCR⁻ CD4⁻ CD8⁻ cells. In the thymic cortex, these T cells will rearrange their TCR genes and differentiate into double positive CD4⁺ CD8⁺ cells (58). It is not completely understood how the division into single positive CD4⁺ and CD8⁺ T cells occurs. However it is believed that the fate of double positive cells is divided between two options; either it dies due to neglect if thymic epithelial cells (TEC) do not interact with it (59), or it interacts with MHC I or MHC II on a TEC initiating its development into a CD4⁺ or CD8⁺ cell (60). The minor portion of T cells that survive thymic selection exit the thymus as single positive CD4⁺ or CD8⁺ T cells expressing cell surface receptors that facilitate their entry into secondary lymphoid organs in the periphery (61) where they recognize antigens. Naïve CD4⁺ T cells or T helper (T_H) cells exert their effector functions mainly through secreting cytokines and chemokines that activate and/or recruit target cells. T_H cells have four (and possible more) distinct fates that are determined by the pattern of signals they receive during their initial interaction with antigen. These four populations are Th1, Th2, Th17 and induced regulatory T cells (iTreg) (62–64).

The immature B cell expresses IgM and IgD antibodies on its surface as it migrate from the bone marrow and enters the **secondary lymphoid organ, the lymph node**. In this organ B cells are activated by T helper (T_H) cells, follicular dendritic cells (FDC), or antigens. Following activation it will start to proliferate. The activation induced (cytidine) deaminase (**AID**) will introduce point mutations on the variable region of the DNA to increase their collective affinity for a particular antigen and any of its close variants (also called somatic hypermutation). AID is also involved in the **class-switch recombination** in which B cells change their expression from IgM to IgG or other isotypes. The B cell will then differentiate into a plasma cell or a memory B cell with a different type of antibody class.

2.2.1 Antibody structure

From the earliest studies of antibody structure it was clear that all antibody domains, whether variable or constant, form compact globular structures with a characteristic fold, termed the immunoglobulin fold (65–68). Each domain consists of a stable arrangement of hydrogen-bonded, anti-parallel β -strands that forms a bilayer structure, which is further stabilized by a disulfide bond between the two layers. Immunoglobulins (Igs) or antibodies (abs) can be divided into five major classes (isotypes): **IgM, IgG, IgA, IgD, and IgE**. The IgG class has been most extensively studied and is also the most abundant in the circulation of normal individuals. The IgG consists of two identical “**light**” chains (**L**) and two identical “**heavy**” chains (**H**) (**Figure 1**). Both the L-chain and the H-chain consist of **variable and constant regions** (V_L, C_L, V_H, and C_H domains). Disulphide bonds (S-S) are covalently linking the four individual chains together. Enzymatic digestion of the molecule results in three fragments, each with a molecule weight of approximately 50kDa. Two of these fragments are identical and are called the **Fab** fragments and the third is called **Fc**.

Each Fab fragment consists of a complete L-chain and the N-terminal half of a H-chain. The Fab fragment retains the antigen binding activity of the parent molecule, although it can only behave as a monovalent antibody. The Fabs are linked to the Fc by the **hinge region**, which varies in length and flexibility in the different antibody classes and isotypes.

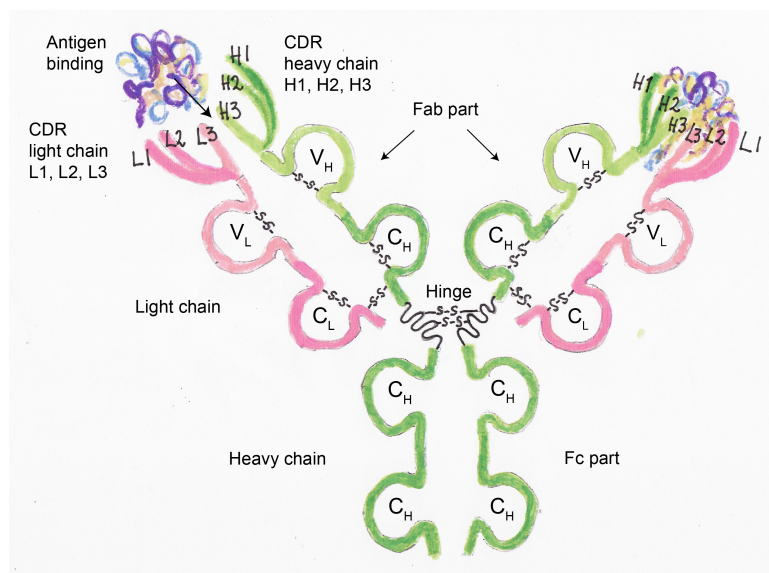


Figure 1. Simplified illustration of an immunoglobulin recognizing its antigen. The IgG consists of two identical “**light**” chains (**L**), colored in pink and two identical “**heavy**” chains (**H**) colored in green.

The antigen binding sites (**paratopes**) are located at the tips of the Fabs. Comparative studies on H-chains of the same class have shown that the sequence of the constant H-chain domains C_{H1} , C_{H2} and C_{H3} remain constant whereas the V_H regions display variability. Just as in the L-chain, the variable region of the H-chain occurs at the N-terminal end of the molecule, is approximately 110 amino acid residues in length and contains hypervariable regions (69,70). The antigen-binding site is confined to the V_L and the V_H regions and these hypervariable regions specify the conformation of the antigen binding site of immunoglobulin molecules (**antigen complementarity regions, CDRs**) (69–71).

2.2.2 Antibody development

The development process starts in the **bone marrow** where DNA encoding the heavy and light chains of the antibodies is rearranged. **Diversity** (D) and **joining** (J) gene segments are recombined (D-J joining) followed by adding a **variable** (V) joining segment to the heavy chain DJ complex. Diversity of the antibodies is produced by the use of different combinations of V, D, and J gene segments in different clones of B cells. The recombination of V and J or V, D, and J, gene segments is mediated by a group of enzymes collectively called VDJ recombinase. The B and T cell-specific **VDJ recombinase** is composed of the **recombinase-activating gene (RAG)-1 and RAG-2** proteins, and they are responsible for these recombination steps (49–51). Additional diversity is produced by sequence changes, and this generates more sequences than are

present in the germline genes. This “junctional” diversity remove or add nucleotides and as a result of these mechanisms, the nucleotide sequence at the site of V(D)J recombination in antibody or TCR molecules made by one clone of B and T cells differs from the sequence at the V(D)J site of antibody or TCR molecules made by every other clone. The junctional sequences encode the amino acids of the CDR3 loop, which are the most variable of the CDRs and the one most important for antigen recognition. Thus, junctional diversity maximizes the variability in the antigen-binding regions of antibodies and TCRs.

3 RHEUMATOID ARTHRITIS

Although the name **rheumatoid arthritis (RA)** was introduced in the 1850s (75), classification criteria were only developed 50 years ago (76,77). RA is a complex autoimmune disorder characterized by inflammatory damage to joints but also by systemic features such as inflammation of blood vessels, lungs, and other tissues. RA is best considered as a clinical syndrome covering several disease subsets (78). These subsets involve several inflammatory cascades (79), which all lead towards a final common pathway.

RA affects about 0.5-1% of the population (80,81), and the prevalence varies geographically (82,83). The disease is common in northern Europe and North America compared with parts of the developing world, such as rural west Africa (84,85). RA is more common in women than in men with a ratio of about 3:1 (86). The primary inflammatory site in RA is the synovial membrane (**Figure 2**) and as RA proceeds hydrops, hyperemia, cartilage destruction and bone erosion and at a later stage malalignment of the connected bones occur.

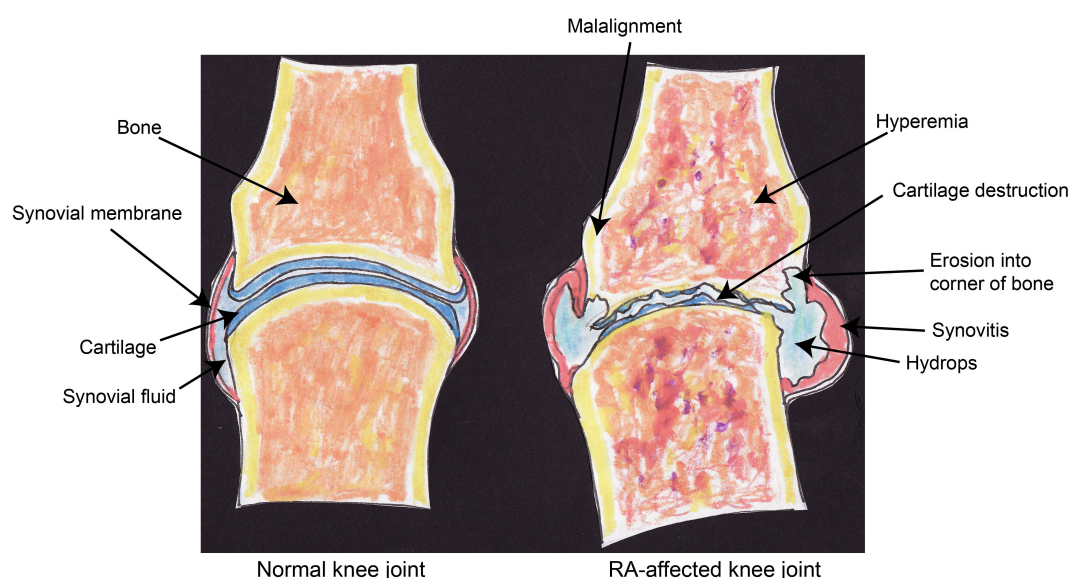


Figure 2. Simplified illustration of a normal joint and an RA-affected joint

3.1 RELEVANT RISK FACTORS FOR DEVELOPING RA

RA is a complex disorder with disease susceptibility being linked to **genetic**, **environmental**, and **immunological factors** as well as to **stochastic events**. The most important genetic risk predisposing to RA is ascribed to the **MHC class II region**, especially to genes encoding HLA-DR molecules. It has been found that all HLA DRB1 alleles that are associated with RA risk (28) encode a conserved sequence of 5 amino acids (positions 70-74) that surrounds the peptide-binding pocket of the antigen-presenting molecule. The presence of this so-called “**shared epitope**” suggests that the molecules containing it might bind the same antigen (29) or alter the peptide affinity that could have a role in promoting autoreactive responses. One of the strongest genes

linked to autoimmunity outside the HLA is the tyrosine phosphatase non-receptor, type 22 (**PTPN22**) (87). The first reported association of PTPN22 with RA was found in 2004 (88). Other genes that have been proposed to be associated with RA are cytotoxic T lymphocyte-associated antigen-4 (**CTLA-4**), peptidyl arginine deaminase, type IV (**PADI4**), and macrophage migration inhibitory factor (**MIF**) (89).

As earlier mentioned not only genes, but also environmental factors are believed to play an important role in the susceptibility to developing RA. One of the highest environmental risk factor in RA is **smoking** (90–92), which like other forms of **bronchial stress** (e.g. exposure to silica) increases the risk of developing RA among patients with susceptible HLA-DR4 alleles (93). The influence of other environmental factors such as **infections**, **hormones**, and **dietary factors** on RA is still unclear.

3.2 AUTOANTIBODIES IN RA

Autoantibodies are commonly observed in autoimmune diseases. **Rheumatoid factor (RF)**, the first autoantibody recognized in RA, was described by Waaler in 1940 (94). Identification and characterization of RF as an autoantibody that binds the Fc portion of IgG was the first direct evidence that autoimmunity might play a role in RA. Long-time studies show that production of RF and other autoantibodies can precede the onset of RA by many years (95). But although they can be detected in 60–80% of RA patients (IgM-RF) (96), there is no clear evidence that RFs are involved in the initial events triggering the disease process of RA. However, it has been shown that RF seropositive patients have more severe clinical disease and complications than seronegative patients included cardiovascular problems (97). RF is not RA-specific and occurs in various infectious diseases (98), other autoimmune diseases most notably Sjögren's syndrome (99) (transiently) and in healthy individuals following immunization (100,101).

Citrullination of proteins is a physiological process that also occurs in inflammation and is therefore not specific for RA. However, presence of **anti-citrullinated protein antibodies (ACPAs)** is highly correlated with classical RA (102,103) whereas RF is more common but less specific since it is present in other diseases. In similarity with RF, the formation of ACPAs precedes the development of RA (95,104–106). ACPAs are found in 70–90% of RA patients and have high disease specificity and sensitivity (107,108). As with RF, they are associated with more erosive RA (78,109,110). ACPAs are locally produced in RA joints, where proteins are citrullinated during the inflammatory process (111). The major citrullinated protein in the joint was found to be fibrin (112). Additionally, various other synovial and non-synovial proteins such as collagen type II, vimentin, nuclear proteins and stress proteins have been shown to be targets of citrullination in vivo (113).

Autoantibodies to collagen type II (CII), a fibrous collagen protein located in the joint cartilage can be detected in serum (7,8,14,114,115) and synovial fluid (7,9,10,116) of patients with RA. The reported incidence of these autoantibodies have varied greatly (3-71%) and this is believed to be dependent on the immunological technique used as well as types and species of native or denatured collagen (114,117–122). Support for a pathogenic role of these antibodies is obtained by that they can precede radiological changes (13,123) or the appearance of rheumatoid factor (RF) (124) and their

frequency has been correlated in RA with the presence of HLA alleles that confer susceptibility to disease (14). Furthermore, injection of human anti-CII antibodies into arthritis susceptible mice induce a mild inflammatory arthritis (12). Similarly, recent studies have also demonstrated the arthritogenicity of plasma/serum from active RA patients in FcγRIIb-deficient mice, and the IgG rich fraction has been identified as the pathogenic factor (11). Moreover, studies have shown that anti-CII positive patients can have a distinct clinical phenotype characterized by an early acute phase response that might be driven by anti-CII containing immune complexes in joint cartilage (125).

3.3 TREATMENT OF RA

Advances in understanding the pathogenesis of the disease have encouraged the development of new therapies, with improved outcomes. To prevent damage to joints, maintain functional status, and decrease pain, treatment guidelines recommend the use of disease-modifying anti-rheumatic drugs (**DMARDs**) immediately after the RA diagnosis has been made (126,127). One of the most common DMARDs, **methotrexate**, remains the first-line medication for moderate-to-severe RA (128). Biological agents have also been introduced that specifically target cytokines in the inflammatory cascade in RA. The primary target of this type of therapy is tumour necrosis factor alpha, TNF-α (129) and there are currently five approved biological (antibody or soluble receptor) for the treatment of RA, which specifically neutralize **TNF-α** (Etanercept, Infliximab, Adalimumab, Certolizumab, and Golimumab). Anakinra, an IL-1 receptor antagonist, and Tocilizumab, a neutralizing monoclonal antibody against human IL-6, are also approved biologicals for the treatment of RA, and in particular Tocilizumab, has been shown to be a good alternative for the treatment of nonresponders to TNF-α inhibitors (130,131). Also Rituximab, a chimeric monoclonal antibody against the CD20 antigen on all B cells except plasma cells, is used as an effective treatment of RA patients (132,133).

4 COLLAGEN INDUCED ARTHRITIS (CIA)

Collagen induced arthritis (CIA) is one of the most common animal models for RA. It can be induced in rodents and non-human primate species by **immunization with CII** emulsified in adjuvant, generally complete Freund's adjuvant (CFA) (134–136).

4.1 CIA IN MICE

The CIA model share several immunological and pathological features with RA (17–20,134,136) such as synovitis, cartilage breakdown and bone deformities but there are also differences between the two as CIA is a time-dependent model induced with CII. CIA, like human RA, is MHC dependent and only occurring in animal strains with the proper class II. The susceptibility is linked to the **H-2^q and H-2^r haplotypes** (27). These haplotypes are also permissive for the induction of arthritis with homologous mouse CII, and development of a strong autoantibody response to CII occurs (137). Furthermore, **transgenic mice** expressing the human HLA-DR4 (DRB1*0401) or DR1 (DRB1*0101) are susceptible to CIA (138–140). Some of these susceptible mouse strains will be discussed in more detail in study III. Both the human MHC II (DRB1) and the mouse H-2^q bind an immunodominant CII peptide located at position 259-273 on triple helical CII (CII259-273) (30,140–142). Immune response to this peptide has also been detected in RA patients (6,143,144). The CII259-273 peptide binds to the DR4*0401 molecule with the major MHC anchors shifted three amino acids as compared with the binding of the same peptide to the H-2^q molecule but the T cell receptor recognition sites are partly shared (140,145,146). Thus, these humanized mouse strains provide us with a CIA model that shares molecular structures of possible critical importance for the human disease.

4.1.1 Anti-CII antibodies in CIA

The need of B cells in CIA has been demonstrated in B cell deficient mice (147) and a strong B cell response to CII occur after CIA induction (23,148,149). Several studies have revealed detailed CII epitope analysis using the technique of **monoclonal antibody production** (23,150–155). To date, the most characterized and dominant CII epitopes recognized by autoantibodies in CIA are denoted **C1, U1, and J1**. Additional CII epitopes have been discovered and are discussed in more detail in study I. The antibodies bind to **conformation-dependent** epitopes at distinct positions along the native molecule (156) and do not crossreact to either denatured CII or to other collagens. The structural importance of the anti-CII recognition has also been shown, as CIA cannot be induced in rats and mice by denatured CII (157,158).

There are several studies demonstrating the pathogenic nature of autoantibodies to CII directly in vivo (55,159–165). These studies show that anti-CII antibodies can induce arthritis in mice. As an example, injection of a cocktail of anti-CII monoclonal antibodies induce an very acute arthritis denoted **collagen antibody induced arthritis (CAIA)** (161,164). There is also evidence that the epitope specificity of the antibodies could have a major role in disease development since it previously was

shown that co-injection of a monoclonal antibody specific for epitope F4, denoted CII-F4 reduced the induction of arthritis in mice. Interestingly, antibodies to the F4 epitope were in fact negatively associated with RA whereas antibodies to epitope C1, J1 and U1, were positively associated (166). Autoantibodies to C1, U1, and J1 are well-characterized and dominates the response in CIA in mice (167,168) and rats (169). Monoclonal antibodies against these major CII epitopes show unique arthritogenic capacities. For instance the monoclonal antibody **CII C1** (discussed in detail in study II) show degradative effects in vitro on cartilage synthesis, and disorganization of fibrils in the extracellular matrix (16). Another pathogenic monoclonal antibody denoted **M2139** (discussed in detail in study I and IV) cause thickening and aggregation of CII fibrils in the extra cellular matrix and abnormal chondrocyte morphology (170). Furthermore, a monoclonal antibody against the U1 epitope, **UL1** induced proteoglycan depletion in vivo independent of inflammation (22), discussed more in study I.

4.1.2 T cell epitope on CII

The T cell epitope on CII was discovered by Michaëlsson et al (30) and consists of a 12 amino acid long sequence GIAGFKGEQGPK located at positions 259-270 on triple helical CII. The CII molecule can be **posttranslationally modified** (at position 264 and 270) by lysine hydroxylation and glycosylation. It was previously shown that the K at position 264 is the key amino acid residue for CII-specific T cell recognition in H-2^q and HLA-DR4 mouse models (32,171). The modifications generate many possible T cell epitopes that seem to be recognized by specific T cell clones (171,172). Most of the T cells activated after immunization with heterologous CII react with the CII259-273 peptide.

4.2 CIA IN NON-HUMAN PRIMATES

CIA can also be induced in non-human primate species, most known and characterized is the rhesus monkey (*Macaca mulatta*). The animals are immunized with CII emulsified in CFA and swelling of joints develops after 3-4 weeks (173). The target tissue of disease onset is the synovium. Removal of the synovium by gene therapy in rhesus monkeys with clinically active CIA abolishes joint inflammation (174). Rhesus monkeys (of Indian/Burmese origin) positive for the Mamu B26 MHC class I marker are resistant to the development of clinical arthritis (175). The resistance is associated with the incapacity to produce an adequate IgM antibody response against the immunizing CII. By selecting animals lacking the Mamu B26 marker, a reproducible induction of the disease at an incidence > 95% can be obtained. CII-specific antibodies of the IgM isotype play a pivotal role in rodent models of CIA (176) and also in rhesus monkeys (177) and common marmosets (178).

Recently a new CIA model was introduced in the New World primate species, the common marmosets (178). This model shares many characteristics with the rhesus monkeys but at the level of histopathology, less cartilage and bone destruction is observed compared with the rhesus monkeys. Anti-CII antibody responses in these two strains will be discussed in study I.

5 COLLAGEN TYPE II (CII)

Collagens are a large group of proteins that can be divided into several subgroups characterized by structure and function. Collagen type II (CII) belongs to the group called **fibril-forming collagens**. The group of fibril-forming collagens includes type I-III, V, and XI collagen. They are all similar in size and, they all contain large triple helical domains with about 1000 amino acids per chain (179). Already in the intracellular space they are going through modification and assembly, events involving post-translational hydroxylation and glycosylation, association of polypeptide chains, and folding of the triple helix. Collagens of this group are first synthesized as procollagen, characterized by non-helical extensions and the N- and C-termini that are then cleaved by specific proteinases after secretion (**Figure 3**). Extracellularly, they will self-assemble into fibrils followed by crosslinking of the fibrils. Another shared characteristic is that each molecule is displaced about one-quarter of its length relative to its nearest neighbor along the axis of the fibril (180).

The collagen triple helix is formed from three identical polypeptide chains that are each coiled around each other to form a right-handed super helix resembling a rope-like rod (181,182). Formation of this super-helical structure is facilitated by the presence of a glycine as every third amino acid. Due to the absence of a side-chain only glycine is small enough to fit in at specific positions in the center of the triple helix where the three chain come together. The amino acid **proline** is frequently in the X-position of the - **Gly-X-Y**- sequence and **4-hydroxyproline** is frequently in the Y-position. The rigidity of these two amino acids favors specific backbone conformations and limits rotation of the polypeptide chains. A regular hydrogen-bonding network between the chains further stabilizes the triple helix. The conformation of the triple helix places the side-chains of amino acids in the X- and Y- positions on the surface of the molecule. This arrangement explains the ability of many collagens to polymerize, since the multiple clusters of hydrophobic and charged side-chains directly self-assemble into precisely ordered structures. The triple helix is relatively **rigid**. In some contexts, the resistance of the molecule to extension or compression is important for the biological function of the protein. In many collagens, the triple helix is interrupted by globular sequences that make the molecule more flexible.

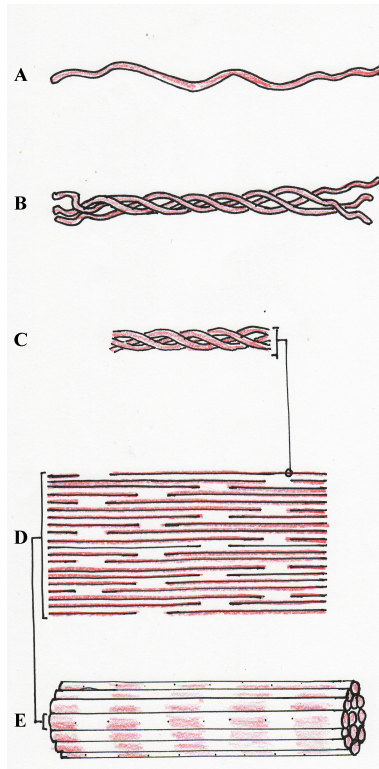


Figure 3 Simplified illustration of how CII self assembles into fibrils and fibers. Three pro α -chains (A) self assemble and the procollagen triple helix forms (B). Specific proteinases cleave the non-helical ends of the triple helix (C) and the triple helical molecule self assemble into fibrils that can covalently crosslink to each other (D). Finally the fibrils aggregate to form CII fiber (E).

6 PRESENT INVESTIGATION

6.1 METHODOLOGIES

Methods used in paper I-IV are described in detail in the respective “Materials and Methods” sections. Here follows an overview of the methods that mainly were used in this thesis with reference to the papers in which they were applied:

Animal experiments (I, III, IV)	Induction and assessment of CIA, organ dissection, serum collection
Circular dichroism (CD) (II)	Analysis of the secondary structure of proteins
Fab preparation (II, IV)	Antibody digestion using papain and purification of Fab fragments
Gel filtration chromatography (I, II)	Purification technique to separate proteins according to size
Monoclonal antibody production (I, IV)	Process by which large quantities of antibodies can be produced by fusion of single antibody producing cells with tumor cells grown in culture
Polymerase chain reaction (PCR) (II, IV)	Amplification of DNA generating thousands to millions copies of a particular DNA sequence
Radioactive proliferation assay (III)	Assay to measure the proliferation rate of cells in vitro by incorporation of ^3H -thymidine into the cell DNA
Recombinant protein production (I-II, IV)	Cloning, expression and purification of recombinant proteins
Sequencing (II, IV)	Determining the nucleotide sequence of a gene using labeled probes
Site-directed mutagenesis (II, IV)	Methods to introduce mutations at specific sites in plasmid DNA
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (I-II)	Analysis of protein size and purity
Solid phase enzyme-linked immunosorbent assay (ELISA) (I-IV)	Analysis of antibody binding capacities to various targets in vitro
Statistical analysis (I-IV)	One-way Anova (Kruskal-Wallis test), Dunn’s multiple comparison tests, Wilcoxon signed rank test, Mann-Whitney test
T cell in vitro assays (III)	Assay based on re-stimulation of cell cultures with various agents, followed by measuring cytokines in the supernatant

X-ray crystallography (II, IV):	
Protein crystallization by hanging and sitting drop vapour diffusion	Technique to generate protein crystals for use in X-ray crystallography
Molecular replacement (MR)	X-ray crystallography method using diffraction data from crystals of a protein in combination with an already solved crystal structure of a homologue for structural determination
Model building and refinement	Computer analysis using the obtained electron density maps to build a first model of the structure. The model is then refined (improved) through statistical adjustment of the atomic coordinates

6.2 STUDY I

Analysis of autoantibodies to the joint specific protein collagen II in experimental and rheumatoid arthritis

This study was undertaken in order to obtain a more comprehensive understanding of CII epitope specific responses in mice and monkeys with CIA and in patients with established RA. The work was based on the generation of a recombinant CII peptide library covering overlapping sequences from the entire triple-helical region of CII. CIA was induced in B10.Q (BALB/c x B10.Q) N2 developing a chronic relapsing disease and in B10.Q developing an acute arthritis. Sera were collected at different disease timepoints and analyzed for autoantibody specificity. Autoantibodies specific for several hitherto unknown CII epitopes were discovered and earlier discovered CII epitopes were confirmed. The epitope predominance varied during the disease course. The specific autoantibody response detected in mice was also apparent in non-human primate species (rhesus monkeys and common marmosets) with CIA. Furthermore, immunodominant CII epitopes with known biological significance in mice were tested for autoantibody reactivity in serum and synovial fluid of patients with established RA. All three investigated CII epitopes (C1, U1, and J1) were detected in a subgroup of patients and interestingly they were all significantly increased in the synovial fluid, in particular autoantibodies against epitope U1 with frequencies reaching close to 70%.

6.3 STUDY II

Crystal structure of an arthritogenic anti-collagen immune complex

Little is known about the structural specificity of a pathogenic autoantibody when interacting with CII. This study was therefore performed to investigate how one of the most pathogenic autoantibodies in mice, CIIC1 recognizes its epitope (C1) on cartilage CII. The CIIC1_{fab} was produced and mixed with the triple-helical C1 peptide and the crystal structure of the complex was determined to 2.2 Å resolution, revealing how antibody and epitope interact in molecular detail. The importance of the observed interactions was further investigated using site directed mutagenesis of a recombinant C1 peptide. The antibody binding to the C1 peptide did not introduce any significant conformational changes in the antibody although the peptide, which retained its typical triple-helical conformation upon antibody binding, penetrated rather deep into the pocket. All three peptide chains forming the epitope were in contact with the antibody. The antibody contacts the CII peptide primarily via germline-encoded residues. Interestingly the antibody clearly recognized the sequence “R-G *hydrophobic*”, a motif that has been suggested to be a consensus recognition sequence in CII epitopes and potentially a recognition site for autoantibodies. The arginine at position 360 (R360) from all three chains contributed significantly to the binding. Its crucial role was further confirmed by site directed mutagenesis, showing that substitution of R360 by alanine completely destroys the interaction between the antibody and the peptide. All six CDRs interacted with the triple-helical peptide. CDRs H2 and L3 contribute most to the area buried upon peptide binding, while CDR L2 contributes less, mainly because the loop residues are located rather far away from the epitope.

6.4 STUDY III

Comparative analysis of collagen type II-specific immune responses during development of collagen-induced arthritis in two B10 mouse strains

In this study we wanted to understand the magnitude and specificity of how CII-specific T cells respond to modified variants of the T cell epitope in CIA, but also how autoantibodies recognize major CII epitopes over the course of arthritis. The specific T and B cell response was investigated in two mouse strains, B10.Q and a humanized transgenic strain B10.DR4.*Ncf1*^{*/*} after CII immunization. Serum and draining lymph nodes were collected at four timepoints during the disease course and used for analysis of the CII-specific T cell as well as antibody response in the two mouse strains. The analyses revealed no clear shift in either antibody specificity or T cell reactivity between the phases in either of the two strains. Instead, the T cell recall response stayed rather constant with dominant response to the non-modified (K264) and the hydroxylated (HOK264) CII259-273 peptide in the humanized mice and to the glycosylated CII259-273 (GalOK264) peptide in the B10.Q mice. One was noted at the initial stage of CIA in B10.Q mice when a relatively equal T cell reactivity to non-modified (K264) and to the galactosylated (GalOK264) was detected. The C1 epitope was the most recognized of the epitopes investigated in both strains. Antibodies to epitope J1 were almost negligible and the concentration of antibodies to epitope U1 was elevated at all timepoints investigated in the humanized mouse model. In addition, we revealed that the T cell response had much higher magnitude in B10.DR4.*Ncf1*^{*/*} compared to B10.Q. Moreover, titers of anti-CII antibodies as well as anti-C1 antibodies were significantly higher in arthritic mice compared with healthy mice, and so was also the antibody response to U1 and J1 in the transgenic mice.

6.5 STUDY IV

Immunoglobulin heavy chain V gene polymorphism controls epitope specific antibody response

It was recently shown that production of anti-CII antibodies, in particular antibodies specific for the J1 epitope, strongly associate with the IgHv locus on mouse chromosome 12 (168). These studies were performed using high resolution mapping in a heterogeneous stock (HS) mouse cohort (183). In order to better understand this finding we investigated the specific anti-CII response to the arthritogenic J1 epitope on CII in detail.

Sequencing of J1-specific monoclonal antibodies revealed a common IgHv gene usage. Comparing this sequence with those encoded by the same gene in all strains comprising the HS, two major polymorphisms were found in the CDR1 domain (S31R and W33T). In order to investigate the impact of these two polymorphisms upon antibody binding a pathogenic monoclonal antibody M2139_{fab} were crystallized together with the triple helical J1 peptide. The crystallized M2139_{fab}-J1 complex confirmed a strong interaction of these two amino acid residues with the triple helical J1 peptide. By modeling, almost all interactions were lost after replacing the two polymorphic sites in the CDR1 region suggesting that these positions are major contributors to the M2139_{fab}-J1 affinity. This was further confirmed using recombinant single chain fragments (ScFv) and site-directed mutagenesis.

The crystal structure was also used to compare the immune complex with the CIIC1_{fab}-C1 antibody complex described earlier (184). We observed clear differences in how the collagen peptides are oriented in the antibody-combining site, with C1 remaining relatively straight and the J1 peptide bending into the pocket. Although the structures of both fab fragments superimpose remarkably well and the CDR sequences show a considerable degree of structural and sequence conservation, none of the crucial interactions with the peptide are conserved. M2139_{fab}-J1 makes only about half as many polar interactions to the J1 peptide than CIIC1 forms with C1. The major recognition motif also markedly differed with M2139_{fab} compared to CII-C1 was detected. The major recognition motif also markedly differed with M2139_{fab} primarily binding to I₂₆A₂₇G₂₈ and CIIC1_{fab} recognizing the R₁₈G₁₉L₂₀.

7 DISCUSSION AND FUTURE PERSPECTIVE

Unraveling CII autoimmunity is an important task in order to understand the basic mechanisms operating in autoimmune diseases such as RA. The research I have performed during my PhD have answered several important questions and provided new insights into these areas but they have of course also raised new questions. I will below outline my views of the result obtained and further suggest how to continue with the research within this field.

One of the purposes of this thesis was to build a more comprehensive understanding of biological significant CII epitopes in CIA and RA. As earlier discoveries were limited to analyses using large CII fragments and specificity of monoclonal antibodies established from the very initiative phase of CIA, we decided to generate a library consisting of 70 overlapping CII peptides covering the entire triple-helical region of CII that are fused with a foldon trimerization domain (185). This library allowed detection of specific epitope binding and revealed the autoantibody response at different time points in a chronic mouse model of CIA. The results indicate that the three CII epitopes C1, U1, and J1, known to be the most common epitopes, become “challenged” by several others that appear as strong or stronger. The majority of the newly identified epitopes are localized on the cyanogen bromide (CB) fragment 11, a region where several important epitopes are located (23). This region seems to more easily attract arthritogenic antibodies. The study further shows the epitope specificity during different stages of the disease. Clearly, we show that some CII epitopes are more frequently recognized in early CIA whereas others appear later in the chronic phase of the disease. This could perhaps depend on the epitope location on CII. Some parts of the CII molecule might not be accessible for binding until cartilage breakdown has been ongoing for a while whereas others are more easily recognized and accessible. If this is the case, different epitopes could be used as markers for different stages of the disease. In order to get a better picture of CII epitope specificities in arthritis it is of course very important to continue discovering the new epitope regions and to study the cartilage degradation effect/s of autoantibody binding to these epitopes. The CII-specific studies were continued by analysis of the epitope specific response (C1, U1, and J1) in serum and synovial fluid from RA patients. A clear overrepresentation of all three CII epitopes was found in the synovial fluid with surprisingly high frequencies of the U1 epitope suggesting that there is local antibody production. To date, we do not have a direct explanation for the elevated levels measured for epitope U1. But these findings could just reflect the importance of investigating CII-specific responses using smaller peptides rather than the entire CII or CB fragments. Earlier results using CII have given inconsistent results partly depending on the not 100% pure CII batches used, leading to contaminants such as other matrix molecules surrounding CII. These molecules could potentially mask the epitopes. So maybe the pure peptide antigens give the more correct response? The library could also be used to reveal CII specific responses in non-human primate species although the assay was partly modified due to crossreactivities to the foldon domain attached at one end of the triple-helical peptides. Although the library generated could be used for many purposes, we suggest introducing an even more comprehensive library including additional CII epitopes that are posttranslationally modified (glycosylated, citrullinated, and hydroxylated).

Moreover it is important to generate even better and more sensitive assays to exactly reveal how RA patients respond to the CII determinants. The way CII peptides are synthesized should also be taken into account to reach the best results.

In the next two studies we wanted to structurally determine how pathogenic autoantibodies recognize native CII in its triple-helical state. These studies were initiated using recombinant CII peptides attached to the foldon domain. However, the resolution reached was not enough to clearly see the interactions between antibody and antigen, thus we used synthetic triple helical CII peptides also for the crystallographic studies. M2139_{fab}-J1 and CIIC1_{fab}-C1 complexes share some common characteristics but have completely different recognition sequences. It has earlier been proposed that the so called “R-G-hydrophobic” motif found in almost all discovered epitopes could be a recognition sequence for autoreactive B cells (23). From the two crystal structures and from finemapping analysis it is clear that anti-CII antibodies do not necessarily share recognition residues in their epitopes. The postulated recognition motif could be of importance for most but not all CII autoantibodies, as indicated by the completely different recognition sequence of antibody M2139. However, although differences exist between the two complexes our finemapping studies still argue for that “R-G”, not necessarily followed by a hydrophobic residue, is a common and crucial recognition motif for at least some anti-CII antibodies. This amino acid selection could partly be explained by the surface exposure of arginine (R) due to its long side chain and charged guanidinium group. We already have projects ongoing that aim at the structure determination of more pathogenic anti-CII antibodies, and hopefully this will increase our knowledge base and understanding of these characteristics in even more detail. In this study we also revealed that contribution of the CDRs to the interaction with the peptides differs: in the M2139 complex VH1 and VL3 provided most to the area buried upon peptide binding, in CIIC1 VH2 and VL3. Although the CII epitopes are not identical it has earlier been shown earlier that anti-CII antibodies commonly derive from the same V-gene family. That could be based on the similarity of CII epitopes with regard to being located on a conserved triple helical molecule with high prevalence of repeated sequences.

The main purpose of this thesis was to describe structural requirements for how autoreactive B cells recognize CII. However, since both B and T cells are important for CIA we undertook one additional study aiming to analyze both CII-specific B and T cell responses. CIA was induced in two mouse strains B10.Q and the humanized DR4.*Ncf1*^{*/*} on a B10 genetic background and autoantibodies and IFN- γ levels were measured at different timepoint during the disease course. We show in this study that both T and B cell responses are clearly present but both the magnitude and specificity of the T cell response differ remarkably between the two strains. The B10.DR4.*Ncf1*^{*/*} shows higher levels of IFN- γ at all phases investigated compared with B10.Q. The reason for this difference is entirely clear but one could suggest that the *Ncf1* mutation has an effect of the results since it regulates susceptibility and severity of CIA (188). Another explanation could be differences in the number of T cells expressing CD4⁺ co-receptors between the two strains. In the humanized mouse model all T cells including CD8⁺, express CD4⁺ co-receptor on the cell membrane leading to that also CD8⁺ T cells could be responsible for the observed response. Therefore the magnitude in T cell responses between these transgenic and non-transgenic mice needs to be optimized

before results can be conclusive. One way to circumvent this bias could be by establishing a DR4 (and human CD4) knock-in mouse instead of using a transgene. This would lead to a more physiological expression of the proteins. When investigating the extent to which T cells recognize posttranslational modifications of CII in DR4 and B10.Q mice during the disease course we revealed that the non-glycosylated CII259-273 peptide was clearly immunodominant in DR4 at all phases investigated whereas the galactosylated epitope dominated in H-2^q expressing mice. Thus the H-2^q mice show more similarities to the CII-specific T cell response observed in human RA (6). The explanation for this biased result is currently unknown but maybe also in this case non-physiological expression of the DR4 transgene might influence which CII peptides that will become immunodominant.

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9 REFERENCES

1. Smolen JS, Aletaha D. Challenges of predicting treatment response in patients with rheumatoid arthritis. *Nat Clin Pract Rheumatol*. 2005 Dec;1(2):62–3.
2. Glocker MO, Guthke R, Kekow J, Thiesen H-J. Rheumatoid arthritis, a complex multifactorial disease: on the way toward individualized medicine. *Med Res Rev*. 2006 Jan;26(1):63–87.
3. Isaacs JD, Ferraccioli G. The need for personalised medicine for rheumatoid arthritis. *Ann. Rheum. Dis*. 2011 Jan;70(1):4–7.
4. Van Baarsen LG, Wijbrandts CA, Rustenburg F, Cantaert T, Van der Pouw Kraan TC, Baeten DL, et al. Regulation of IFN response gene activity during infliximab treatment in rheumatoid arthritis is associated with clinical response to treatment. *Arthritis Res. Ther*. 2010;12(1):R11.
5. Van der Pouw Kraan TCTM, Wijbrandts CA, Van Baarsen LGM, Voskuyl AE, Rustenburg F, Baggen JM, et al. Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: assignment of a type I interferon signature in a subpopulation of patients. *Ann. Rheum. Dis*. 2007 Aug;66(8):1008–14.
6. Snir O, Bäcklund J, Boström J, Andersson I, Kihlberg J, Buckner JH, et al. Multifunctional T cell reactivity with native and glycosylated type II collagen in rheumatoid arthritis. *Arthritis Rheum*. 2012 Aug;64(8):2482–8.
7. Clague RB, Moore LJ. IgG and IgM antibody to native type II collagen in rheumatoid arthritis serum and synovial fluid. Evidence for the presence of collagen-anticollagen immune complexes in synovial fluid. *Arthritis Rheum*. 1984;27(12):1370–7.
8. Terato K, Shimozuru Y, Katayama K, Takemitsu Y, Yamashita I, Miyatsu M, et al. Specificity of antibodies to type II collagen in rheumatoid arthritis. *Arthritis Rheum*. 1990;33(10):1493–500.
9. Tarkowski A, Klareskog L, Carlsten H, Herberts P, Koopman WJ. Secretion of antibodies to types I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum*. 1989;32(9):1087–92.
10. Londei M, Savill CM, Verhoef A, Brennan F, Leech ZA, Duan V, et al. Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proceedings of the National Academy of Sciences of the United States of America*. 1989;86(2):636–40.
11. Petkova SB, Konstantinov KN, Sproule TJ, Lyons BL, Awwami MA, Roopenian DC. Human antibodies induce arthritis in mice deficient in the low-affinity inhibitory IgG receptor FcγRIIB. *J Exp Med*. 2006 Feb 20;203(2):275–80.
12. Wooley PH, Luthra HS, Singh SK, Huse AR, Stuart JM, David CS. Passive transfer of arthritis to mice by injection of human anti-type II collagen antibody. *Mayo Clin Proc*. 1984;59(11):737–43.

13. Cook AD, Rowley MJ, Mackay IR, Gough A, Emery P. Antibodies to type II collagen in early rheumatoid arthritis. Correlation with disease progression. *Arthritis and rheumatism*. 1996;39(10):1720–7.
14. Cook AD, Stockman A, Brand CA, Tait BD, Mackay IR, Muirden KD, et al. Antibodies to type II collagen and HLA disease susceptibility markers in rheumatoid arthritis. *Arthritis Rheum*. 1999;42(12):2569–76.
15. Amirahmadi SF, Whittingham S, Crombie DE, Nandakumar KS, Holmdahl R, Mackay IR, et al. Arthritogenic anti-type II collagen antibodies are pathogenic for cartilage-derived chondrocytes independent of inflammatory cells. *Arthritis and rheumatism*. 2005;52(6):1897–906.
16. Amirahmadi SF, Pho MH, Gray RE, Crombie DE, Whittingham SF, Zuasti BB, et al. An arthritogenic monoclonal antibody to type II collagen, CII-C1, impairs cartilage formation by cultured chondrocytes. *Immunology and cell biology*. 2004;82(4):427–34.
17. Brunsberg U, Gustafsson K, Jansson L, Michaelsson E, Ahrlund-Richter L, Pettersson S, et al. Expression of a transgenic class II Ab gene confers susceptibility to collagen-induced arthritis. *Eur J Immunol*. 1994;24(7):1698–702.
18. Nandakumar KS, Holmdahl R. Antibody-induced arthritis: disease mechanisms and genes involved at the effector phase of arthritis. *Arthritis research & therapy*. 2006;8(6):223.
19. Myers LK, Rosloniec EF, Cremer MA, Kang AH. Collagen-induced arthritis, an animal model of autoimmunity. *Life Sci*. 1997;61(19):1861–78.
20. Cremer MA, Rosloniec EF, Kang AH. The cartilage collagens: a review of their structure, organization, and role in the pathogenesis of experimental arthritis in animals and in human rheumatic disease. *J. Mol. Med*. 1998 Mar;76(3-4):275–88.
21. Croxford AM, Whittingham S, McNaughton D, Nandakumar KS, Holmdahl R, Rowley MJ. Collagen type II-specific antibodies induce cartilage damage independent of inflammation. *Arthritis Rheum*. 2012 Dec 12;
22. Nandakumar KS, Bajtner E, Hill L, Bohm B, Rowley MJ, Burkhardt H, et al. Arthritogenic antibodies specific for a major type II collagen triple-helical epitope bind and destabilize cartilage independent of inflammation. *Arthritis and rheumatism*. 2008;58(1):184–96.
23. Schulte S, Unger C, Mo JA, Wendler O, Bauer E, Frischholz S, et al. Arthritis-related B cell epitopes in collagen II are conformation-dependent and sterically privileged in accessible sites of cartilage collagen fibrils. *The Journal of biological chemistry*. 1998;273(3):1551–61.
24. Moder KG, Luthra HS, Kubo R, Griffiths M, David CS. Prevention of collagen induced arthritis in mice by treatment with an antibody directed against the T cell receptor alpha beta framework. *Autoimmunity*. 1992;11(4):219–24.

25. Ranges GE, Sriram S, Cooper SM. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* 1985 Sep 1;162(3):1105–10.
26. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ. Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40. *Science.* 1993 Sep 3;261(5126):1328–30.
27. Wooley PH, Luthra HS, Stuart JM, David CS. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J Exp Med.* 1981;154(3):688–700.
28. Stastny P. Association of the B-Cell Alloantigen DRw4 with Rheumatoid Arthritis. *New England Journal of Medicine.* 1978;298(16):869–71.
29. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* 1987;30(11):1205–13.
30. Michaelsson E, Andersson M, Engstrom A, Holmdahl R. Identification of an immunodominant type-II collagen peptide recognized by T cells in H-2q mice: self tolerance at the level of determinant selection. *Eur J Immunol.* 1992;22(7):1819–25.
31. Backlund J, Carlsen S, Hoger T, Holm B, Fugger L, Kihlberg J, et al. Predominant selection of T cells specific for the glycosylated collagen type II epitope (263-270) in humanized transgenic mice and in rheumatoid arthritis. *Proc Natl Acad Sci U S A.* 2002;99(15):9960–5.
32. Batsalova T, Dzhambazov B, Merky P, Bäcklund A, Bäcklund J. Breaking T cell tolerance against self type II collagen in HLA-DR4-transgenic mice and development of autoimmune arthritis. *Arthritis Rheum.* 2010 Jul;62(7):1911–20.
33. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* 1989;54 Pt 1:1–13.
34. Matzinger P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 1994;12:991–1045.
35. Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm.* 2010;2010.
36. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell.* 2006 Feb 24;124(4):783–801.
37. Medzhitov R, Janeway C Jr. Innate immunity. *N. Engl. J. Med.* 2000 Aug 3;343(5):338–44.
38. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature.* 2007 Sep 27;449(7161):419–26.
39. Sallusto F, Lanzavecchia A. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J. Exp. Med.* 1999 Feb 15;189(4):611–4.

40. Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* 2000 Feb 7;191(3):411–6.
41. Clark GJ, Angel N, Kato M, López JA, MacDonald K, Vuckovic S, et al. The role of dendritic cells in the innate immune system. *Microbes Infect.* 2000 Mar;2(3):257–72.
42. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* 2010 Jul;10(7):467–78.
43. Godfrey DI, Rossjohn J. New ways to turn on NKT cells. *J. Exp. Med.* 2011 Jun 6;208(6):1121–5.
44. Rus H, Cudrici C, Niculescu F. The role of the complement system in innate immunity. *Immunol. Res.* 2005;33(2):103–12.
45. Morgan BP, Marchbank KJ, Longhi MP, Harris CL, Gallimore AM. Complement: central to innate immunity and bridging to adaptive responses. *Immunol. Lett.* 2005 Mar 15;97(2):171–9.
46. Neumann E, Barnum SR, Turner IH, Echols J, Fleck M, Judex M, et al. Local production of complement proteins in rheumatoid arthritis synovium. *Arthritis Rheum.* 2002 Apr;46(4):934–45.
47. Linton SM, Morgan BP. Complement activation and inhibition in experimental models of arthritis. *Mol. Immunol.* 1999 Oct;36(13-14):905–14.
48. Banda NK, Kraus D, Vondracek A, Huynh LH, Bendele A, Holers VM, et al. Mechanisms of effects of complement inhibition in murine collagen-induced arthritis. *Arthritis Rheum.* 2002 Nov;46(11):3065–75.
49. Banda NK, Kraus DM, Muggli M, Bendele A, Holers VM, Arend WP. Prevention of collagen-induced arthritis in mice transgenic for the complement inhibitor complement receptor 1-related gene/protein y. *J. Immunol.* 2003 Aug 15;171(4):2109–15.
50. Dreja H, Annenkov A, Chernajovsky Y. Soluble complement receptor 1 (CD35) delivered by retrovirally infected syngeneic cells or by naked DNA injection prevents the progression of collagen-induced arthritis. *Arthritis Rheum.* 2000 Aug;43(8):1698–709.
51. Goodfellow RM, Williams AS, Levin JL, Williams BD, Morgan BP. Soluble complement receptor one (sCR1) inhibits the development and progression of rat collagen-induced arthritis. *Clin. Exp. Immunol.* 2000 Jan;119(1):210–6.
52. Hietala MA, Jonsson I-M, Tarkowski A, Kleinau S, Pekna M. Complement deficiency ameliorates collagen-induced arthritis in mice. *J. Immunol.* 2002 Jul 1;169(1):454–9.

53. Wang Y, Rollins SA, Madri JA, Matis LA. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc. Natl. Acad. Sci. U.S.A.* 1995 Sep 12;92(19):8955–9.
54. Woodruff TM, Strachan AJ, Dryburgh N, Shiels IA, Reid RC, Fairlie DP, et al. Antiarthritic activity of an orally active C5a receptor antagonist against antigen-induced monarticular arthritis in the rat. *Arthritis Rheum.* 2002 Sep;46(9):2476–85.
55. Hietala MA, Nandakumar KS, Persson L, Fahlen S, Holmdahl R, Pekna M. Complement activation by both classical and alternative pathways is critical for the effector phase of arthritis. *Eur J Immunol.* 2004;34(4):1208–16.
56. Banda NK, Takahashi K, Wood AK, Holers VM, Arend WP. Pathogenic complement activation in collagen antibody-induced arthritis in mice requires amplification by the alternative pathway. *J. Immunol.* 2007 Sep 15;179(6):4101–9.
57. Cooper MD, Alder MN. The evolution of adaptive immune systems. *Cell.* 2006 Feb 24;124(4):815–22.
58. Von Boehmer H. Developmental biology of T cells in T cell-receptor transgenic mice. *Annu. Rev. Immunol.* 1990;8:531–56.
59. Kisielow P, Teh HS, Blüthmann H, Von Boehmer H. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature.* 1988 Oct 20;335(6192):730–3.
60. Cosgrove D, Chan SH, Waltzinger C, Benoist C, Mathis D. The thymic compartment responsible for positive selection of CD4⁺ T cells. *Int. Immunol.* 1992 Jun;4(6):707–10.
61. Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. *Annu. Rev. Immunol.* 1992;10:561–91.
62. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986 Apr 1;136(7):2348–57.
63. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity.* 2006 Jun;24(6):677–88.
64. Sakaguchi S. Naturally Arising CD4⁺ Regulatory T Cells for Immunologic Self-Tolerance and Negative Control of Immune Responses. *Annual Review of Immunology.* 2004;22(1):531–62.
65. Poljak RJ. X-ray crystallographic studies of immunoglobulins. *Contemp Top Mol Immunol.* 1973;2:1–26.
66. Poljak RJ, Amzel LM, Chen BL, Phizackerley RP, Saul F. The three-dimensional structure of the fab' fragment of a human myeloma immunoglobulin at 2.0-angstrom resolution. *Proc. Natl. Acad. Sci. U.S.A.* 1974 Sep;71(9):3440–4.

67. Epp O, Colman P, Fehlhammer H, Bode W, Schiffer M, Huber R, et al. Crystal and molecular structure of a dimer composed of the variable portions of the Bence-Jones protein REI. *Eur. J. Biochem.* 1974 Jun 15;45(2):513–24.
68. Segal DM, Padlan EA, Cohen GH, Rudikoff S, Potter M, Davies DR. The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. *Proc. Natl. Acad. Sci. U.S.A.* 1974 Nov;71(11):4298–302.
69. Wu TT, Kabat EA. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. *J. Exp. Med.* 1970 Aug 1;132(2):211–50.
70. Kabat EA, Wu TT. Attempts to locate complementarity-determining residues in the variable positions of light and heavy chains. *Ann. N. Y. Acad. Sci.* 1971 Dec 31;190:382–93.
71. Kehoe JM, Capra JD. Phylogenetic aspects of immunoglobulin variable region diversity. *Contemp Top Mol Immunol.* 1974;3:143–59.
72. Tonegawa S. Somatic generation of antibody diversity. , Published online: 14 April 1983; | doi:10.1038/302575a0. 1983 Apr 14;302(5909):575–81.
73. Oettinger MA, Schatz DG, Gorka C, Baltimore D. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science.* 1990 Jun 22;248(4962):1517–23.
74. Schatz DG, Oettinger MA, Baltimore D. The V(D)J recombination activating gene, RAG-1. *Cell.* 1989 Dec 22;59(6):1035–48.
75. Storey GO, Comer M, Scott DL. Chronic arthritis before 1876: early British cases suggesting rheumatoid arthritis. *Ann. Rheum. Dis.* 1994 Sep;53(9):557–60.
76. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31(3):315–24.
77. Diagnostic Criteria for Rheumatoid Arthritis. *Ann Rheum Dis.* 1959 Mar;18(1):49–53.
78. Van der Helm-van Mil AHM, Huizinga TWJ. Advances in the genetics of rheumatoid arthritis point to subclassification into distinct disease subsets. *Arthritis Res. Ther.* 2008;10(2):205.
79. Van Oosterhout M, Bajema I, Levarht EWN, Toes REM, Huizinga TWJ, Van Laar JM. Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive rheumatoid arthritis and anti-cyclic citrullinated peptide-negative rheumatoid arthritis. *Arthritis Rheum.* 2008 Jan;58(1):53–60.
80. Gabriel SE. The epidemiology of rheumatoid arthritis. *Rheum. Dis. Clin. North Am.* 2001 May;27(2):269–81.
81. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature.* 2003 May 15;423(6937):356–61.

82. Costenbader KH, Chang S-C, Laden F, Puett R, Karlson EW. Geographic Variation in Rheumatoid Arthritis Incidence among Women in the United States. *Arch Intern Med.* 2008 Aug 11;168(15):1664–70.
83. Biver E, Beague V, Verloop D, Mollet D, Lajugie D, Baudens G, et al. Low and stable prevalence of rheumatoid arthritis in northern France. *Joint Bone Spine.* 2009 Oct;76(5):497–500.
84. Kalla AA, Tikly M. Rheumatoid arthritis in the developing world. *Best Pract Res Clin Rheumatol.* 2003 Oct;17(5):863–75.
85. Rothschild BM, Woods RJ, Rothschild C, Sebes JL. Geographic distribution of rheumatoid arthritis in ancient North America: implications for pathogenesis. *Semin. Arthritis Rheum.* 1992 Dec;22(3):181–7.
86. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev.* 2005 Mar;4(3):130–6.
87. Gregersen PK. Gaining insight into PTPN22 and autoimmunity. *Nat. Genet.* 2005 Dec;37(12):1300–2.
88. Begovich AB, Carlton VEH, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet.* 2004 Aug;75(2):330–7.
89. Orozco G, Rueda B, Martin J. Genetic basis of rheumatoid arthritis. *Biomed. Pharmacother.* 2006 Dec;60(10):656–62.
90. Vessey MP, Villard-Mackintosh L, Yeates D. Oral contraceptives, cigarette smoking and other factors in relation to arthritis. *Contraception.* 1987 May;35(5):457–64.
91. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann. Rheum. Dis.* 2003 Sep;62(9):835–41.
92. Silman AJ, Newman J, MacGregor AJ. Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. *Arthritis Rheum.* 1996 May;39(5):732–5.
93. Symmons DP, Bankhead CR, Harrison BJ, Brennan P, Barrett EM, Scott DG, et al. Blood transfusion, smoking, and obesity as risk factors for the development of rheumatoid arthritis: results from a primary care-based incident case-control study in Norfolk, England. *Arthritis Rheum.* 1997 Nov;40(11):1955–61.
94. Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles. 1939. *APMIS.* 2007 May;115(5):422–438; discussion 439.

95. Nielen MMJ, Van Schaardenburg D, Reesink HW, Van de Stadt RJ, Van der Horst-Bruinsma IE, De Koning MHMT, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* 2004 Feb;50(2):380–6.
96. Nell VPK, Machold KP, Stamm TA, Eberl G, Heinzl H, Uffmann M, et al. Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. *Ann. Rheum. Dis.* 2005 Dec;64(12):1731–6.
97. Liang KP, Maradit-Kremers H, Crowson CS, Snyder MR, Thorneau TM, Roger VL, et al. Autoantibodies and the Risk of Cardiovascular Events. *J Rheumatol.* 2009 Nov 1;36(11):2462–9.
98. Williams RC Jr. Hypothesis: rheumatoid factors are antiidiotypes related to bacterial or viral Fc receptors. *Arthritis Rheum.* 1988 Sep;31(9):1204–7.
99. Nowak UM, Newkirk MM. Rheumatoid factors: good or bad for you? *Int. Arch. Allergy Immunol.* 2005 Oct;138(2):180–8.
100. AHO K, KONTTINEN A, RAJASALMI M, WAGER O. Transient appearance of the rheumatoid factor in connection with prophylactic vaccinations. *Acta Pathol Microbiol Scand.* 1962;56:478–9.
101. Carson DA, Chen PP, Fox RI, Kipps TJ, Jirik F, Goldfien RD, et al. Rheumatoid factor and immune networks. *Annu. Rev. Immunol.* 1987;5:109–26.
102. Van Venrooij WJ, Zendman AJW, Pruijn GJM. Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis. *Autoimmun Rev.* 2006 Nov;6(1):37–41.
103. Mimori T. Clinical significance of anti-CCP antibodies in rheumatoid arthritis. *Intern. Med.* 2005 Nov;44(11):1122–6.
104. Aho K, Palosuo T, Heliövaara M, Knekt P, Alha P, Von Essen R. Antifilaggrin antibodies within “normal” range predict rheumatoid arthritis in a linear fashion. *J. Rheumatol.* 2000 Dec;27(12):2743–6.
105. Kurki P, Aho K, Palosuo T, Heliövaara M. Immunopathology of rheumatoid arthritis. Antikeratin antibodies precede the clinical disease. *Arthritis Rheum.* 1992 Aug;35(8):914–7.
106. Rantapaa-Dahlqvist S, De Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.* 2003;48(10):2741–9.
107. Schellekens GA, Visser H, De Jong BA, Van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum.* 2000 Jan;43(1):155–63.
108. Suzuki K, Sawada T, Murakami A, Matsui T, Tohma S, Nakazono K, et al. High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. *Scand. J. Rheumatol.* 2003;32(4):197–204.

109. Kroot EJ, De Jong BA, Van Leeuwen MA, Swinkels H, Van den Hoogen FH, Van't Hof M, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum.* 2000 Aug;43(8):1831–5.
110. Im CH, Kang EH, Ryu HJ, Lee JH, Lee EY, Lee YJ, et al. Anti-cyclic citrullinated peptide antibody is associated with radiographic erosion in rheumatoid arthritis independently of shared epitope status. *Rheumatol. Int.* 2009 Jan;29(3):251–6.
111. Reparon-Schuijt CC, Van Esch WJ, Van Kooten C, Schellekens GA, De Jong BA, Van Venrooij WJ, et al. Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. *Arthritis Rheum.* 2001 Jan;44(1):41–7.
112. Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, Nogueira L, Vincent C, Senshu T, et al. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *J Immunol.* 2001;166(6):4177–84.
113. Koivula M-K, Aman S, Karjalainen A, Hakala M, Risteli J. Are there autoantibodies reacting against citrullinated peptides derived from type I and type II collagens in patients with rheumatoid arthritis? *Ann. Rheum. Dis.* 2005 Oct;64(10):1443–50.
114. Clague RB, Shaw MJ, Holt PJ. Incidence of serum antibodies to native type I and type II collagens in patients with inflammatory arthritis. *Ann Rheum Dis.* 1980;39(3):201–6.
115. Clague RB, Morgan K, Reynolds I, Williams HJ. The prevalence of serum IgG antibodies to type II collagen in American patients with rheumatoid arthritis. *Br J Rheumatol.* 1994;33(4):336–8.
116. Rönnelid J, Lysholm J, Engström-Laurent A, Klareskog L, Heyman B. Local anti-type II collagen antibody production in rheumatoid arthritis synovial fluid. Evidence for an HLA-DR4-restricted IgG response. *Arthritis Rheum.* 1994 Jul;37(7):1023–9.
117. Steffen C, Timpl R. Antigenicity of collagen and its application in the serological investigation of rheumatoid arthritis sera. *Int. Arch. Allergy Appl. Immunol.* 1963;22:333–49.
118. Michaeli D, Fudenberg HH. Antibodies to collagen in patients with emphysema. *Clin. Immunol. Immunopathol.* 1974 Nov;3(2):187–92.
119. Andriopoulos NA, Mestecky J, Miller EJ, Bradley EL. Antibodies to native and denatured collagens in sera of patients with rheumatoid arthritis. *Arthritis Rheum.* 1976 Jun;19(3):613–7.
120. Greenbury CL, Skingle J. Anti-cartilage antibody. *J. Clin. Pathol.* 1979 Aug;32(8):826–31.

121. Beard HK, Lea DJ, Ryvar R. Anomalous reactions in the haemagglutination assay for anti-collagen antibodies: studies on patients with rheumatoid arthritis or chronic low back pain. *J. Immunol. Methods.* 1979;31(1-2):119–28.
122. Clague RB, Shaw MJ, Holt PJ. Incidence and correlation between serum IgG and IgM antibodies to native type II collagen in patients with chronic inflammatory arthritis. *Ann Rheum Dis.* 1981;40(1):6–10.
123. Mullazehi M, Wick MC, Klareskog L, Van Vollenhoven R, Ronnelid J. Anti-type II collagen antibodies are associated with early radiographic destruction in rheumatoid arthritis. *Arthritis Research & Therapy.* 2012;14(3):R100.
124. Fujii K, Tsuji M, Kitamura A, Murota K. The diagnostic significance of anti-type II collagen antibody assay in rheumatoid arthritis. *Int Orthop.* 1992;16(3):272–6.
125. Mullazehi M, Mathsson L, Lampa J, Ronnelid J. High anti-collagen type-II antibody levels and induction of proinflammatory cytokines by anti-collagen antibody-containing immune complexes in vitro characterise a distinct rheumatoid arthritis phenotype associated with acute inflammation at the time of disease onset. *Ann Rheum Dis.* 2007;66(4):537–41.
126. Guidelines AC of RS on RA. Guidelines for the management of rheumatoid arthritis: 2002 Update. *Arthritis & Rheumatism.* 2002;46(2):328–46.
127. Neogi T, Aletaha D, Silman AJ, Naden RL, Felson DT, Aggarwal R, et al. The 2010 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Rheumatoid Arthritis. *Arthritis Rheum.* 2010 Sep;62(9):2582–91.
128. Saag KG, Teng GG, Patkar NM, Anuntiyo J, Finney C, Curtis JR, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis Rheum.* 2008 Jun 15;59(6):762–84.
129. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet.* 1989 Jul 29;2(8657):244–7.
130. Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet.* 2008 Mar 22;371(9617):987–97.
131. Nakashima Y, Kondo M, Harada H, Horiuchi T, Ishinishi T, Jojima H, et al. Clinical evaluation of tocilizumab for patients with active rheumatoid arthritis refractory to anti-TNF biologics: tocilizumab in combination with methotrexate. *Mod Rheumatol.* 2010 Aug;20(4):343–52.
132. Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum.* 2006 Sep;54(9):2793–806.

133. Emery P, Fleischmann R, Filipowicz-Sosnowska A, Schechtman J, Szczepanski L, Kavanaugh A, et al. The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. *Arthritis Rheum.* 2006 May;54(5):1390–400.
134. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. *The Journal of experimental medicine.* 1977;146(3):857–68.
135. Yoo TJ, Kim SY, Stuart JM, Floyd RA, Olson GA, Cremer MA, et al. Induction of arthritis in monkeys by immunization with type II collagen. *The Journal of experimental medicine.* 1988;168(2):777–82.
136. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature.* 1980;283(5748):666–8.
137. Holmdahl R, Andersson M, Enander I, Goldschmidt T, Jansson L, Larsson P, et al. Nature of the type II collagen autoimmunity in mice susceptible to collagen-induced arthritis. *International reviews of immunology.* 1988;4(1):49–64.
138. Rosloniec EF, Brand DD, Myers LK, Whittington KB, Gumanovskaya M, Zaller DM, et al. An HLA-DR1 transgene confers susceptibility to collagen-induced arthritis elicited with human type II collagen. *J. Exp. Med.* 1997 Mar 17;185(6):1113–22.
139. Rosloniec EF, Brand DD, Myers LK, Esaki Y, Whittington KB, Zaller DM, et al. Induction of autoimmune arthritis in HLA-DR4 (DRB1*0401) transgenic mice by immunization with human and bovine type II collagen. *J. Immunol.* 1998 Mar 15;160(6):2573–8.
140. Andersson EC, Hansen BE, Jacobsen H, Madsen LS, Andersen CB, Engberg J, et al. Definition of MHC and T cell receptor contacts in the HLA-DR4-restricted immunodominant epitope in type II collagen and characterization of collagen-induced arthritis in HLA-DR4 and human CD4 transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 1998 Jun 23;95(13):7574–9.
141. Fugger L, Rothbard JB, Sonderstrup-McDevitt G. Specificity of an HLA-DRB1*0401-restricted T cell response to type II collagen. *Eur. J. Immunol.* 1996 Apr;26(4):928–33.
142. Brand DD, Myers LK, Terato K, Whittington KB, Stuart JM, Kang AH, et al. Characterization of the T cell determinants in the induction of autoimmune arthritis by bovine alpha 1(II)-CB11 in H-2q mice. *J. Immunol.* 1994 Mar 15;152(6):3088–97.
143. Bäcklund J, Carlsen S, Höger T, Holm B, Fugger L, Kihlberg J, et al. Predominant selection of T cells specific for the glycosylated collagen type II epitope (263-270) in humanized transgenic mice and in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 2002 Jul 23;99(15):9960–5.

144. Kim HY, Kim WU, Cho ML, Lee SK, Youn J, Kim SI, et al. Enhanced T cell proliferative response to type II collagen and synthetic peptide CII (255-274) in patients with rheumatoid arthritis. *Arthritis Rheum.* 1999 Oct;42(10):2085–93.
145. Rosloniec EF, Whittington KB, Brand DD, Myers LK, Stuart JM. Identification of MHC class II and TCR binding residues in the type II collagen immunodominant determinant mediating collagen-induced arthritis. *Cell. Immunol.* 1996 Aug 25;172(1):21–8.
146. Kjellen P, Brunsberg U, Broddefalk J, Hansen B, Vestberg M, Ivarsson I, et al. The structural basis of MHC control of collagen-induced arthritis; binding of the immunodominant type II collagen 256-270 glycopeptide to H-2Aq and H-2Ap molecules. *Eur J Immunol.* 1998;28(2):755–67.
147. Svensson L, Jirholt J, Holmdahl R, Jansson L. B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clinical and experimental immunology.* 1998;111(3):521–6.
148. Holmdahl R, Jansson L, Gullberg D, Rubin K, Forsberg PO, Klareskog L. Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II. *Clin. Exp. Immunol.* 1985 Dec;62(3):639–46.
149. Larsson P, Kleinau S, Holmdahl R, Klareskog L. Homologous type II collagen-induced arthritis in rats. Characterization of the disease and demonstration of clinically distinct forms of arthritis in two strains of rats after immunization with the same collagen preparation. *Arthritis Rheum.* 1990 May;33(5):693–701.
150. Milstein C, Cuello AC. Hybrid hybridomas and their use in immunohistochemistry. *Nature.* 1983 Oct 6;305(5934):537–40.
151. Holmdahl R, Mo JA, Jonsson R, Karlstrom K, Scheynius A. Multiple epitopes on cartilage type II collagen are accessible for antibody binding in vivo. *Autoimmunity.* 1991;10(1):27–34.
152. Karlsson R, Mo JA, Holmdahl R. Binding of autoreactive mouse anti-type II collagen antibodies derived from the primary and the secondary immune response investigated with the biosensor technique. *Journal of immunological methods.* 1995;188(1):63–71.
153. Mo JA, Bona CA, Holmdahl R. Variable region gene selection of immunoglobulin G-expressing B cells with specificity for a defined epitope on type II collagen. *Eur J Immunol.* 1993;23(10):2503–10.
154. Mo JA, Scheynius A, Nilsson S, Holmdahl R. Germline-encoded IgG antibodies bind mouse cartilage in vivo: epitope- and idiotype-specific binding and inhibition. *Scandinavian journal of immunology.* 1994;39(2):122–30.
155. Mo JA, Holmdahl R. The B cell response to autologous type II collagen: biased V gene repertoire with V gene sharing and epitope shift. *J Immunol.* 1996;157(6):2440–8.

156. Burkhardt H, Holmdahl R, Deutzmann R, Wiedemann H, Von der Mark H, Goodman S, et al. Identification of a major antigenic epitope on CNBr-fragment 11 of type II collagen recognized by murine autoreactive B cells. *European journal of immunology*. 1991;21(1):49–54.
157. Holmdahl R, Vingsbo C, Malmström V, Jansson L, Holmdahl M. Chronicity of arthritis induced with homologous type II collagen (CII) in rats is associated with anti-CII B-cell activation. *J. Autoimmun.* 1994 Dec;7(6):739–52.
158. Stuart JM, Townes AS, Kang AH. Nature and specificity of the immune response to collagen in type II collagen-induced arthritis in mice. *J Clin Invest.* 1982 Mar;69(3):673–83.
159. Johansson AC, Hansson AS, Nandakumar KS, Bäcklund J, Holmdahl R. IL-10-deficient B10.Q mice develop more severe collagen-induced arthritis, but are protected from arthritis induced with anti-type II collagen antibodies. *J. Immunol.* 2001 Sep 15;167(6):3505–12.
160. Svensson L, Nandakumar KS, Johansson A, Jansson L, Holmdahl R. IL-4-deficient mice develop less acute but more chronic relapsing collagen-induced arthritis. *Eur. J. Immunol.* 2002 Oct;32(10):2944–53.
161. Nandakumar KS, Andren M, Martinsson P, Bajtner E, Hellstrom S, Holmdahl R, et al. Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcγRIIB. *Eur J Immunol.* 2003;33(8):2269–77.
162. Nandakumar KS, Svensson L, Holmdahl R. Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. *The American journal of pathology.* 2003;163(5):1827–37.
163. Nandakumar KS, Backlund J, Vestberg M, Holmdahl R. Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. *Arthritis research & therapy.* 2004;6(6):R544–50.
164. Nandakumar KS, Holmdahl R. Efficient promotion of collagen antibody induced arthritis (CAIA) using four monoclonal antibodies specific for the major epitopes recognized in both collagen induced arthritis and rheumatoid arthritis. *J. Immunol. Methods.* 2005 Sep;304(1-2):126–36.
165. Stuart JM, Dixon FJ. Serum transfer of collagen-induced arthritis in mice. *J. Exp. Med.* 1983 Aug 1;158(2):378–92.
166. Burkhardt H, Koller T, Engstrom A, Nandakumar KS, Turnay J, Kraetsch HG, et al. Epitope-specific recognition of type II collagen by rheumatoid arthritis antibodies is shared with recognition by antibodies that are arthritogenic in collagen-induced arthritis in the mouse. *Arthritis and rheumatism.* 2002;46(9):2339–48.

167. Bajtner E, Nandakumar KS, Engstrom A, Holmdahl R. Chronic development of collagen-induced arthritis is associated with arthritogenic antibodies against specific epitopes on type II collagen. *Arthritis research & therapy*. 2005;7(5):R1148–57.
168. Förster M, Raposo B, Ekman D, Klaczewska D, Popovic M, Nandakumar KS, et al. Genetic control of antibody production during collagen induced arthritis development in heterogeneous stock mice. *Arthritis Rheum*. [Internet]. 2012 Aug 8 [cited 2012 Aug 22]; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22886420>
169. Wernhoff P, Unger C, Bajtner E, Burkhardt H, Holmdahl R. Identification of conformation-dependent epitopes and V gene selection in the B cell response to type II collagen in the DA rat. *International immunology*. 2001;13(7):909–19.
170. Amirahmadi SF, Whittingham S, Crombie DE, Nandakumar KS, Holmdahl R, Mackay IR, et al. Arthritogenic anti-type II collagen antibodies are pathogenic for cartilage-derived chondrocytes independent of inflammatory cells. *Arthritis and rheumatism*. 2005;52(6):1897–906.
171. Corthay A, Bäcklund J, Broddefalk J, Michaëlsson E, Goldschmidt TJ, Kihlberg J, et al. Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. *Eur. J. Immunol*. 1998 Aug;28(8):2580–90.
172. Corthay A, Bäcklund J, Holmdahl R. Role of glycopeptide-specific T cells in collagen-induced arthritis: an example how post-translational modification of proteins may be involved in autoimmune disease. *Ann. Med*. 2001 Oct;33(7):456–65.
173. Vierboom MP, Jonker M, Bontrop RE, 't Hart B. Modeling human arthritic diseases in nonhuman primates. *Arthritis research & therapy*. 2005;7(4):145–54.
174. Goossens PH, Schouten GJ, Hart BA 't, Bout A, Brok HPM, Kluin PM, et al. Feasibility of Adenovirus-Mediated Nonsurgical Synovectomy in Collagen-Induced Arthritis-Affected Rhesus Monkeys. *Human Gene Therapy*. 1999 May;10(7):1139–49.
175. Bakker NP, Van Erck MG, Otting N, Lardy NM, Noort RC, 't Hart BA, et al. Resistance to collagen-induced arthritis in a nonhuman primate species maps to the major histocompatibility complex class I region. *J. Exp. Med*. 1992 Apr 1;175(4):933–7.
176. Zheng B, Zhang X, Guo L, Han S. IgM plays an important role in induction of collagen-induced arthritis. *Clin. Exp. Immunol*. 2007 Sep;149(3):579–85.
177. Vierboom MPM, Jonker M, Tak PP, 't Hart BA. Preclinical models of arthritic disease in non-human primates. *Drug Discovery Today*. 2007 Apr;12(7–8):327–35.
178. Vierboom MP, Breedveld E, Kondova I, 't Hart BA. Collagen-induced arthritis in common marmosets: a new nonhuman primate model for chronic arthritis. *Arthritis research & therapy*. 2010;12(5):R200.

179. Van der Rest M, Garrone R. Collagen family of proteins. *FASEB J.* 1991 Oct;5(13):2814–23.
180. Hulmes DJ, Wess TJ, Prockop DJ, Fratzl P. Radial packing, order, and disorder in collagen fibrils. *Biophys J.* 1995 May;68(5):1661–70.
181. Fleischmajer R, Kuhn K, Olsen BR. Structure, molecular biology, and pathology of collagen / edited by Raul Fleischmajer, Bjorn R. Olsen, and Klaus Kuhn. New York, N.Y. :: New York Academy of Sciences; 1990.
182. Engel J, Prockop DJ. The Zipper-Like Folding of Collagen Triple Helices and the Effects of Mutations that Disrupt the Zipper. *Annual Review of Biophysics and Biophysical Chemistry.* 1991;20(1):137–52.
183. Ahlqvist E, Ekman D, Lindvall T, Popovic M, Förster M, Hultqvist M, et al. High-resolution mapping of a complex disease, a model for rheumatoid arthritis, using heterogeneous stock mice. *Hum. Mol. Genet.* 2011 Aug 1;20(15):3031–41.
184. Dobritzsch D, Lindh I, Uysal H, Nandakumar KS, Burkhardt H, Schneider G, et al. Crystal structure of an arthritogenic anticollagen immune complex. *Arthritis Rheum.* 2011 Dec;63(12):3740–8.
185. Frank S, Kammerer RA, Mechling D, Schulthess T, Landwehr R, Bann J, et al. Stabilization of short collagen-like triple helices by protein engineering. *J. Mol. Biol.* 2001 May 18;308(5):1081–9.
186. Meier S, Güthe S, Kiefhaber T, Grzesiek S. Foldon, the natural trimerization domain of T4 fibrin, dissociates into a monomeric A-state form containing a stable beta-hairpin: atomic details of trimer dissociation and local beta-hairpin stability from residual dipolar couplings. *J. Mol. Biol.* 2004 Dec 3;344(4):1051–69.
187. Stetefeld J, Frank S, Jenny M, Schulthess T, Kammerer RA, Boudko S, et al. Collagen stabilization at atomic level: crystal structure of designed (GlyProPro)₁₀foldon. *Structure.* 2003;11(3):339–46.
188. Hultqvist M, Olofsson P, Holmberg J, Bäckström BT, Tordsson J, Holmdahl R. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the *Ncf1* gene. *PNAS.* 2004 Aug 24;101(34):12646–51.