From undifferentiated arthritis to rheumatoid arthritis: epidemiology, immunology and early intervention

Henrike Gillet-van Dongen

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‘On ne voit bien qu’avec le coeur. L’essentiel est invisible pour les yeux.’

Antoine de Saint-Exupéry (1900 - 1944)

Contents

CHAPTER 1

Introduction

Introduction

Arthritis

The clinical diagnosis of arthritis is characterised by warm, painful and swollen joints in one or more areas. The most common chronic inflammatory joint syndrome is rheumatoid arthritis, which affects approximately 1% of the population (1). In rheumatoid arthritis, the arthritis eventually leads to destruction of the joint. Distinguishing rheumatoid arthritis from other types of arthritis can be difficult. In the 1950s, a committee of the American Rheumatism Association (ARA) categorised arthritis based on expert opinions, epidemiologic surveys and clinical cases. Diagnostic criteria for possible, probable and classical or definite rheumatoid arthritis were proposed (2). In 1987 the criteria for rheumatoid arthritis were reassessed (3). These criteria are still used to define distinct research populations. ***Table 1*** shows the criteria for probable rheumatoid arthritis from

Table 1. Criteria for rheumatoid arthritis and probable rheumatoid arthritis

No.	Criterion
1	Morning stiffness.
2	Pain on motion or tenderness in at least one joint (observed by a physician).
3	Swelling (soft tissue thickening or fluid, not bony overgrowth alone) in at least one joint (observed by a physician).
4	Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms between the two joint involvements may not be more than 3 months).
5	Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of midphalangeal, metacarpophalangeal or metatarsophalangeal joints is acceptable without absolute symmetry). Terminal phalangeal joint involvement will not satisfy this criterion.
6	Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces or in juxta-articular regions.
7	X-ray changes typical of rheumatoid arthritis (which must include at least bony decalcification localized to or greatest around the involved joints and not just degenerative changes). Degenerative changes do not exclude patients from any group classified as rheumatoid arthritis.
8	Positive agglutination test - demonstration of the "rheumatoid factor" by any method which, in two laboratories, has been positive in not over 5% of normal controls - or positive streptococcal agglutination test.
9	Poor mucin precipitate from synovial fluid (with shreds and cloudy solution).
10	Characteristic histologic changes in synovial membrane with three or more of the following: marked villous hypertrophy; proliferation of superficial synovial cells often with palisading; marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form "lymphoid nodules"; deposition of compact fibrin, either on surface or interstitially; foci of cell necrosis.
11	Characteristic histologic changes in nodules showing granulomatous foci with central zones of cell necrosis, surrounded by proliferated fixed cells, and peripheral fibrosis and chronic inflammatory cell infiltration, predominantly perivascular.

(A) Diagnostic criteria for probable rheumatoid arthritis (2). This diagnosis requires three of the criteria. In at least one of the criteria number 1 through 5 the joint signs or symptoms must be continuous for at least six weeks.

No.	Criterion	Definition
1	Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2	Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3	Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4	Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5	Rheumatoid nodules	Subcutaneous nodules, over bony prominences, on extensor surfaces, or in juxtaarticular regions, observed by a physician
6	Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7	Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

(B) 1987 Criteria for the classification of acute arthritis of rheumatoid arthritis (3). For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

1958 and for rheumatoid arthritis from 1987. If the arthritis does not fulfil any criteria belonging to a disease description by the American College of Rheumatology and other diagnoses involving arthritis are ruled out, you are left with an undifferentiated arthritis. This undifferentiated arthritis can be an early stage of rheumatoid arthritis which has not been recognised yet as such. To study amongst others the epidemiology of arthritis, early arthritis clinics were set up throughout the world. In Leiden, an Early Arthritis Clinic was started in 1993. From the first 1000 patients who were included 37% was classified as having an undifferentiated arthritis (4). After 1 year, the diagnosis of the patients who presented with an undifferentiated arthritis was reassessed (5). About 30% of these patients had no signs of arthritis after 1 year. However, 28% of the patients were recognised within 1 year to have rheumatoid arthritis.

Monitoring arthritis

Rheumatoid arthritis is characterised by progressive destruction of the joints, either slowly or rapidly. These destructions eventually lead to disability. To monitor efficacy of treatments different methods were developed to evaluate the intensity of a (destructive) rheumatoid arthritis. The main methods that are used in the next chapters are described in this paragraph.

The disease activity score (DAS) consists of both objective and subjective items. It was developed using a discriminant analysis based on the decision of rheumatologists to start or switch an anti-rheumatic drug in combination with parallel blind clinical assessments of research nurses (6;7). The DAS is calculated as follows:

DAS = 0.53938 * sqrt (Ritchie score)
+ 0.06465 * (number of swollen joints)
+ 0.330 * ln (erythrocyte sedimentation rate)
+ 0.00722 * (patient global assessment)

In the Ritchie articular index 53 joints are scored for tenderness. Forty-four joints are scored for swelling (8). For most rheumatologists a DAS of more than 2,4 means an active disease state that is high enough to intensify medication (9). A DAS of 1,6 is used as a cut off for remission (10). To simplify the calculation of the DAS for daily practice, substitutes were developed. The original DAS with the mentioned 4 variables was reduced to a DAS with three variables in which the patient global assessment value was replaced by 0,224 (7). A modified DAS that included 28-joint counts instead of 44 (8) and a simplified disease activity index were validated as well (11). In studies described in this thesis the original DAS was used.

A more objective method for measuring the destructiveness of rheumatoid arthritis is scoring the radiographic joint damage. There are different scoring methods to evaluate radiographic joint damage. In the projects described in this thesis the Sharp-van der Heijde modification scoring method was used. This method focuses on the small joints in the hands, the wrists and the feet. Joint space narrowing is scored in 42 joints, and the distribution of erosions is scored in 44 joints, as is depicted in ***Figure 1*** (12).

The health assessment questionnaire (HAQ), arthritis impact measurement scales (AIMS), rheumatoid arthritis disease activity index (RADAI) and modifications on them are patient centered assessments focussing on the impact of the arthritic disease in daily life (13-16). For example, performance of physical activities is graded by the patient, and the general well being and disease activity are rated on visual analogue scales (VAS) from 1 to 10 by the patient or the physician.

Evidence for early treatment

There is no curative treatment for rheumatoid arthritis. Over the past twenty years, the treatment of rheumatoid arthritis has significantly changed with the introduction of disease-modifying anti-rheumatic drugs (DMARDs) and later on the introduction of biologicals. Before 1994 most patients who were diagnosed with RA started with a non-steroid anti-inflammatory drug (NSAID). NSAIDs give symptom reduction, but are not thought to interfere with the underlying disease process in contrast to DMARDs. Moreover, it was shown that patients with RA who started with a DMARD as soon as

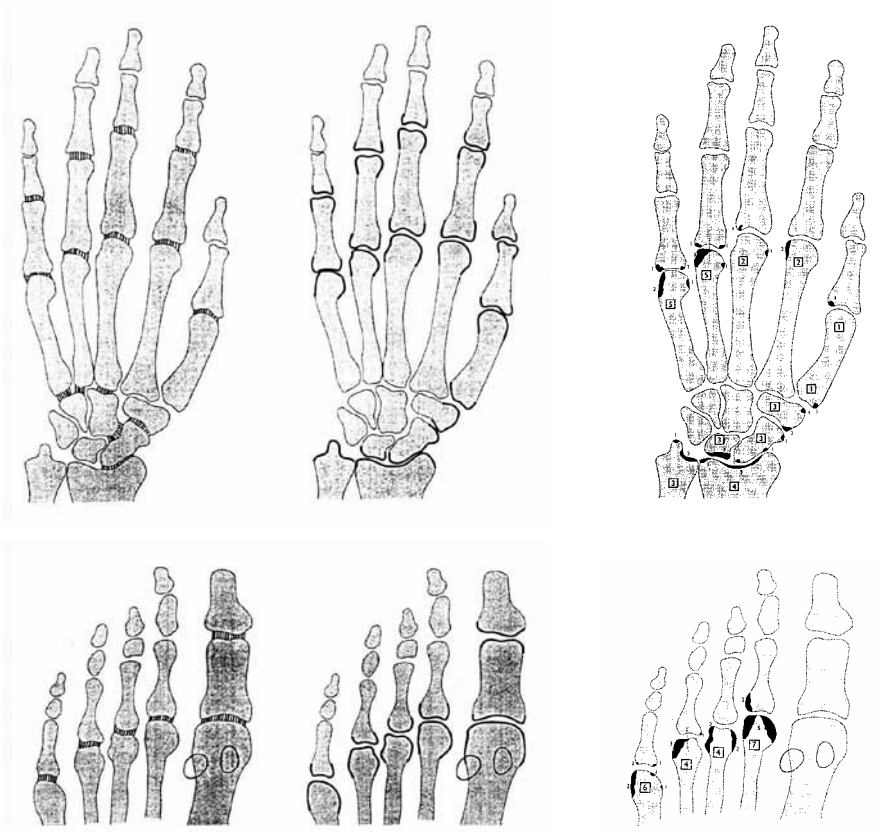

Figure 1. Scoring of hands and feet according to the Sharp-van der Heijde scoring method
Joints and sites scored for joint space narrowing (left panel), joints and surfaces of the joints scored for erosions (middle panel), examples of scoring erosions according to the joint surface involved (right panel) with the van der Heijde modification for hands and feet. The small numbers indicate how an erosion is scored and the numbers in the boxes give the total score for that joint. Reproduced from *Van der Heijde DMFM. Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability. Bailliere's Clinical Rheumatology 1996* (12).

the diagnosis was made had less disease activity and less radiographic joint damage or progression of it compared to patients who first started with NSAIDs, and the best results were yielded in the first year of treatment (17-19). After 10 years, the beneficial effects of immediate treatment with DMARDs was even reflected in a lower need for joint surgery (20). Taking this knowledge into account, it was proposed that recognizing rheumatoid arthritis in an early phase is crucial to the disease outcome on the long term, a so-called window of opportunity ***(Figure 2)***. Treatment strategies have only been developed for patients in the clinical phase who are recognised to have rheumatoid arthritis. The window of opportunity actually lies in recognising individuals who are at risk for developing rheumatoid arthritis in the preclinical phase and in recognising

patients with an undifferentiated arthritis who actually have rheumatoid arthritis, but have no joint destruction yet.

Pathophysiology

Rheumatoid arthritis is classified as an autoimmune disease, in which leucocytes attack the joints, leading to inflammation and resulting in destruction of the joint. Different factors are thought to be involved in the pathophysiology of rheumatoid arthritis. Genetic susceptibility and environmental factors can create the conditions in which harmful T cell responses are activated that are also able to provide help to B cells with subsequent production of antibodies. Past decades different parts of this cascade have been under investigation.

Genetic background

The influence of a genetic component for rheumatoid arthritis is emphasised in a three- to fourfold higher concordance percentage of disease in monozygotic twins compared to dizygotic twins and the total genetic contribution to rheumatoid arthritis is estimated to be 50-60% (21-24). A wide variety of candidate genes has been investigated for their influence on susceptibility to and severity of rheumatoid arthritis. The most prominent genetic risk factor found so far are the human leucocyte antigen (HLA) class II molecules. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis is the shared epitope hypothesis (25). The initiation of an immune response requires T cell activation, and such activation requires the presence of antigen presented by HLA class II molecules on antigen presenting cells. At the level of protein structure, certain HLA-DRB1 alleles share an amino acid sequence in the beta-sheet of the peptide-binding groove of the HLA molecule, the shared epitope. It is thought that the shared epitope containing HLA-molecules are important for the presentation of arthritogenic antigens. The shared epitopes, QRRAA (DR1), QKRAA (DR4) and RRRAA (DR10), are not only associated with susceptibility to rheumatoid arthritis, but also with a more progressive disease course (26;27). The contribution of the presence of the shared epitope counts for 30% of the genetic aspects of rheumatoid arthritis (28). However, the putative peptides that fit in the groove and are directly involved in the cause of rheumatoid arthritis have not been discovered so far.

T cells

The involvement of HLA class II molecules and the presence of joint antigen-directed T cells suggest a role for CD4+ T cells (29). After arising from bone marrow stem cell precursors, progenitor cells migrate to the thymus, where they will be maturated into T cells. In the thymus the T cells learn to distinguish self from non-self in the context of HLA

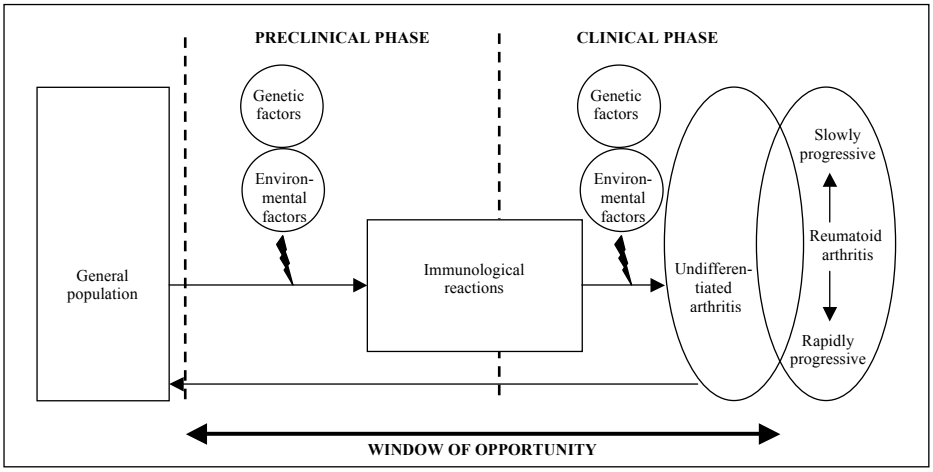

Figure 2. Working model for the disease course of arthritis

molecules. Firstly, those T cells that recognise self-HLA molecules are positively selected. Secondly, the negative selection process is said to eliminate the auto-reactive T cells.

Roughly, there are two types of T cells. Cytotoxic T cells, characterised by CD8 expression, recognise antigens in the context of HLA class I molecules, which are present on almost all nucleated cells. HLA class I molecules are loaded with intracellular proteins. If the CD8+ T cell is activated, it kills the antigen presenting cell by lysis. During these actions, pro-inflammatory cytokines like interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha and interleukin-2 (IL-2) are released.

T helper cells express CD4 and recognise antigens in the context of HLA class II molecules. Only antigen presenting cells like dendritic cells, monocytes, macrophages and B cells can process extracellular proteins to present them in HLA class II to CD4+ T cells. If the T cell is activated, it in turn activates a B cell that then starts to produce isotype-switched antibodies. This process is characterised by the release of IL-4 and IL-10.

However, this does not explain why regulatory or suppressor T cells exist and why or how autoimmune diseases develop. Different hypotheses about the origin of autoimmunity have been described varying from genetic defects involving the selection processes in the thymus to danger models in which damaged tissue releases danger signals that work as a costimulation factor for activating an auto-reactive process (30-33). The functional existence of regulatory T cells was first demonstrated in nude mice in which infusion with CD25+CD4+ T cells after transfer of CD25-CD4+ T cells prevented development of autoimmune diseases (34). CD25 is the alpha chain of the IL-2 receptor. It is highly expressed on activated T cells in the early phase of activation, often described as the CD25 bright cells in flowcytometric analysis, whereas regulatory T cells show an intermediate expression (35;36). Other markers like cytotoxic T lymphocyte antigen-4

(CTLA-4), glucocorticoid induced TNF receptor (GITR) and foxP3 have been used to describe regulatory T cells, but are also not specific. The regulatory T cell response is characterised by the release of anti-inflammatory cytokines like IL-10 and tumour growth factor (TGF)-beta (37;38). Thus, T cell-mediated immunoregulation does likely play a role in immunologic self-tolerance but exact characterisation of the regulatory T cells remains difficult, especially in a human setting.

Autoantibodies

The presence of autoantibodies is also a reason to consider rheumatoid arthritis an autoimmune disease. The positive predictive value of the presence of certain antibodies aims at another phase of the immune response that may play a role in the pathophysiology of rheumatoid arthritis. Rheumatoid factor (RF) is an antibody that is directed to the Fc part of immunoglobulins. The presence of IgM-RF is part of the 1987 criteria for rheumatoid arthritis. However, RF is also detected in other autoimmune diseases and healthy individuals. For diagnosing rheumatoid arthritis, the sensitivity of RF ranges from 60 to 70%; the specificity from 80 to 90% (39).

Anti-citrullinated protein antibodies (ACPAs) are much more predictive for rheumatoid arthritis. In the Leiden EAC, 93% of the patients with ACPAs who presented with undifferentiated arthritis were diagnosed with rheumatoid arthritis within 3 years (40). Furthermore, patients with ACPA-positive rheumatoid arthritis had more erosive disease than patients who had no ACPAs. Citrullination facilitates the degradation process in a cell and is a normal physiologic process in the presence of inflammation (41). A calcium-ion influx leads to activation of the peptidylarginine deiminase (PAD) enzyme, which converts arginine into citrulline. The formation of antibodies against citrullinated proteins is not physiological and is mainly found in rheumatoid arthritis (42). ACPAs can be present up to 14 years upon diagnosing rheumatoid arthritis (43;44). During the last years it has become clear that the genetic and environmental risk factors for ACPA-positive and ACPA-negative disease differ. Moreover, the histology differs and, as stated above, the clinical outcome differs. This has led to the awareness that subclassification of rheumatoid arthritis in ACPA-positive and ACPA-negative disease is appropriate (45-47).

Outline of this thesis

The research projects described in this thesis are divided into two themes: part I involves mainly epidemiological and clinical aspects of patients with undifferentiated arthritis and part II involves immunological aspects of patients with rheumatoid arthritis.

The aim in **part I** of this thesis is to describe the epidemiology of undifferentiated arthritis, to gather more evidence for early intervention, and to predict disease outcome. In the Leiden EAC 37% of the patients who presented with arthritis were classified as having an undifferentiated arthritis (4). In **chapter 2**, the incidence of undifferentiated arthritis

in several early arthritis registries in other parts of the world is described. Some patients with rheumatoid arthritis present with a full blown rheumatoid arthritis while others present themselves with clinically an undifferentiated arthritis. To characterise this different onset of disease, the long-term outcome of patients with such a 'slow onset' were compared to the long-term outcome of patients who present with rheumatoid arthritis. The results are described in **chapter 3**. In rheumatoid arthritis evidence for justification of early aggressive treatment is accumulating. Starting treatment for rheumatoid arthritis in patients with undifferentiated arthritis, the window of opportunity, has not been performed before. The PROMPT study was the first randomised controlled trial in which patients with undifferentiated arthritis were treated with a DMARD, methotrexate. The results of the PROMPT-study are presented in **chapter 4**. In the PROMPT-study, the DAS-score was used to evaluate the disease activity in patients with undifferentiated arthritis. Based on the DAS-score the intensity of the study medication was maintained or raised. However, this score system was designed for patients with rheumatoid arthritis. In **chapter 5**, the DAS in patients with undifferentiated arthritis is discussed, investigated and validated. Not all patients who were included in the PROMPT study had a very early stage of rheumatoid arthritis. Before a patient is exposed to a treatment with methotrexate or another DMARD or even more, a combination of DMARDs, the indication for such a treatment should be carefully evaluated. Actually, you would like to predict which patient with undifferentiated arthritis will benefit from an early treatment and who will go spontaneously in remission or will never develop the clinical syndrome of rheumatoid arthritis. Therefore, a rule to predict disease outcome in patients with recent-onset undifferentiated arthritis was developed and validated in the PROMPT study. This rule is presented in **chapter 6**. ACPAs have a strong predictive value in predicting the probability of having a very early stage of rheumatoid arthritis in patients presenting with undifferentiated arthritis. Having identified an individual with an indication for treatment with second-line anti-rheumatic drugs, you are left with a palette of DMARDs and combinations of DMARDs. Tailor-made treatments for each stage of disease focused on individual needs would be the best. In **chapter 7** the response to methotrexate treatment is related to the pre-treatment serum levels of ACPA in patients with undifferentiated arthritis.

In **part II**, the accent lies on the immunological background of rheumatoid arthritis and possibilities for intervention. Biologicals, like anti-TNF-alpha, have had a major impact on the outcome of rheumatoid arthritis. However, it is unclear how TNF-alpha influences the immune response in patients with rheumatoid arthritis. In **chapter 8** the effect of anti-TNF-alpha on regulating the immune response in patients with rheumatoid arthritis is described. In understanding and identifying the underlying immunological changes in healthy individuals to processes resulting in rheumatoid arthritis, T-cell responses against a human cartilage protein, HC-gp39, and in particular the presence of regulatory T cells are investigated in **chapter 9**. As HC-gp39 seemed to be a usefull target in an attempt to

change the pro-inflammatory response in patients with rheumatoid arthritis, a phase I cohort study was performed by others (48). T cells derived from patients included in this study were analysed for their immunological response. However, the results were not conclusive. In **chapter 10** the results of this analysis and of this thesis are summarized and discussed.

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CHAPTER 2

Undifferentiated arthritis - Disease course assessed in several inception cohorts

K.N. Verpoort
H. van Dongen
C.F. Allaart
R.E.M. Toes
F.C. Breedveld
T.W.J. Huizinga

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Abstract

The prognosis of patients with undifferentiated arthritis (UA) may vary from self-limited to severe destructive rheumatoid arthritis (RA). Because early aggressive treatment might offer an effective means to slow disease progression in RA, it is important to identify UA patients who will develop RA and treat them as early as possible. At the same time, inappropriate treatment of patients with a more benign disease course should be avoided. Here, an overview is given of the characteristics and numbers of patients with UA who evolve into RA.

UA is defined as any arthritis that has the potential for a persistent course, without fulfilling the classification criteria for specific rheumatic disorders. To compare endpoints in the different databases, the 1987 ACR criteria for RA were used.

In the nine databases employing a similar definition for undifferentiated arthritis, the proportion of patients with UA that evolved into RA within 1 year varied from 6% to 55%. These differences arise in large part from differences in the inclusion criteria and in the definitions used for UA and RA. The data from the various cohorts support a hypothesis that a considerable proportion of UA patients are actually patients with RA in a very early stage. Controlled intervention studies with early antirheumatic treatment in these patients are mandatory in order to provide further insight into the natural course of UA and to define a treatment strategy that will successfully slow or prevent disease progression.

Introduction

Several studies have indicated a beneficial effect of the early treatment of rheumatoid arthritis (RA) to achieve a less severe disease course or even to induce remission (1-3). The possible extra therapeutic benefit attainable in this early period in the disease has been called the "window of opportunity". Since the presentation pattern of RA varies widely, it has been suggested that the treatment should be started as early as possible, even before patients fulfil the American College of Rheumatology (ACR) criteria for RA (4). Ideally, knowledge of prognostic factors in patients with undifferentiated arthritis (UA) will allow the identification of those patients who will develop RA, so that the inappropriate treatment of patients who will not develop RA can be avoided. For this it is also necessary to know the natural course of UA. The present review will attempt to describe the natural course of UA as reported in early arthritis cohorts.

The first problem encountered in the search for the percentage of patients presenting with UA who will develop RA is the fact that UA is a non-validated description of a phenotype. In clinical practice, all cases of arthritis that cannot be classified in one of the accepted categories are referred to as *e causa ignota* or "undifferentiated". For inclusion in early arthritis cohorts, various definitions and criteria have been used for the early phase of arthritis, which makes it difficult to compare the composition of the different study groups. 'Early arthritis', 'early RA', and 'undifferentiated arthritis' are terms that are currently in use to describe either arthritis that might evolve into RA or that has been diagnosed early after onset of arthritis or even early in the disease course of definite RA. Therefore, patients with UA are in general seen as those patients with the potential for development of persistent inflammatory arthritis, including RA, but in whom a recognized clinical pattern does not (yet) exist. In 1958 the American Rheumatism Association (ARA) identified criteria for 'probable rheumatoid arthritis' (5) as a distinction from classical RA, but these criteria only define a subgroup of patients generally referred to as having UA.

In this review, defining RA according to the classification criteria also has disadvantages from a scientific viewpoint. The ACR criteria for RA were developed to identify patients with established RA, and not for diagnostic purposes. In clinical practice, it is of great relevance to distinguish patients on prognostic items such as persistent arthritis or destructive arthritis. On the other hand, all intervention studies to date have been based on fulfilment of the ACR criteria, and evidence that adequate treatment changes the course of disease as well as the prognosis is available only in patients who meet the ACR criteria. Therefore, notwithstanding the imperfect definitions of the phenotype for clinical practice, it is important to assess what proportion of UAcases progress to RA, as defined by the ACR criteria.

Inception cohorts

Early RA databases and their inclusion criteria are listed in ***Table 1***. The databases marked by an asterisk have included and described patients with UA. Only the latter databases will be discussed. The other databases include 'early RA' patients who fulfilled the 1987 ACR criteria for established RA.

In Finland an early arthritis cohort was started in 1975 (6). Adults with one or more swollen joints and a symptom duration of less than 6 months were referred to the hospital in Heinola. Fortythree percent of the patients from this cohort had non-specific arthritis, defined as probable RA according to the 1958 ARA criteria or arthritis not falling within any

Table 1. Early RA databases

Study group	Inclusion criteria	Study strategy and characteristics	N	Reference
Heinola Cohort/ Rheumatism Foudation Hospital Cohort (Finland) *	≥ 1 swollen joints disease duration ≤ 6 months age ≥ 16 years	prospective cohort referred by phycisians of several health centres and hospitals follow-up after 1, 3, 8, 15, 20 and 25 years	442	(6)
Norfolk Arthritis Register (UK) *	early inflammatory polyarthritis age ≥ 16 years ≥ 2 swollen joints symptom duration ≥ 4 weeks onset after January 1989	referred from GP and local rheumatologists yearly follow-up for at least 5 yrs patient visited at home		(10;22)
Leeds (UK) *	undifferentiated arthritis of the hands symptom duration < 12 months	patients from the Leeds Early Arthritis Clinic (n=1877) pyramid treatment strategy	97	(12)
Duesseldorf (Germany)*	rheumatic symptoms duration ≤ 1 year age > 15 years	2-year prospective cohort study referred by GPs, internist, orthopaedic physicians	320	(13)
Austrian Early Arthritis Registry *	inflammatory arthritis with ≥ 2 clinical criteria and ≥ 1 laboratory criterion duration of symptoms < 12 weeks	referred by GPs and internists to participating rheumatologists multi-centre (country-wide) every 3 months questionnaires		(14;16)
Wichita Arthritis Centre (USA) *	undifferentiated polyarthritis syndrome or RA (ACR'87 criteria) disease duration ≤ 2 years	half of patients self-referred follow-up at least 13 months	506 (RA) 638 (UA)	(17)
ESPOIR Cohort Study (France) *	certain or probable clinical diagnosis of RA UA that may develop into RA duration of symptoms < 6 months age 18-70 years ≥ 2 inflammatory joints for the past 6 weeks no DMARD use prior to inclusion	800 patients from the community 10 yrs follow-up		(18)
Amsterdam (The Netherlands) *	≥ 2 swollen joints disease duration < 3 years	Patients from an early arthritis clinic	203	(19)

Study group	Inclusion criteria	Study strategy and characteristics	N	Reference
Leiden Early Arthritis Clinic (The Netherlands) *	any arthritis confirmed by rheumatologist symptom duration < 2 years no DMARD use prior to inclusion	referred by GPs follow-up at 2 weeks, 3 months and yearly		(20)
EURIDISS-Oslo (Norway)	RA (ACR'87 criteria) age 20-70 years disease duration ≤ 4 yrs	Norwegian part of international collaborative research effort follow-up at 1, 2 and 5 years	238	(23)
French Early Arthritis Cohort	RA (ACR'87 criteria) RA diagnosis < 1 year no DMARD use prior to inclusion	multi-centre referred from primary care follow-up 10 year		(18)
GIARA Registry Study Group (Italy)	RA (ACR'87 criteria)	aggressive RA registry	706	(24)
Jyäskylä Cohort (1983-1985) (Finland)	newly diagnosed RA (ARA'58 criteria)	follow-up 18-24 months	58	(6;25)
Jyäskylä Cohort (1988-1989) (Finland)	definite RA (ARA'58 criteria) and ≥ 2 criteria (ESR>20mm/hour, ≥ 6 joints with active RA, duration morning stiffness > 45 minutes) age 18-80 years symptom duration < 1 year	randomised, double blind, placebo controlled study on treatment with sulfasalazine follow-up at 4, 8, 12, 24 and 48 weeks	80	(6;26)
Central Finland RA database	(newly) diagnosed RA according to physician	all new patients with RA are referred to Jyäskylä Central Hospital	>2000	(6)
Helsinki Cohort (Finland)	RA (ACR'87 or revised ACR'87 criteria) symptom duration < 2 year no DMARD use prior to inclusion	prospective study on early aggressive therapy referred from primary care or private outpatients clinics	150	(6;27)
FIN-RACo study (Finland)	RA (ACR'87 criteria) symptom duration < 2 year age 18-65 year, ≥ 3 swollen joints and three of: ESR>28, CRP>19, morning stiffness.>29min, >5 swollen joints, >10 tender joints	multi-centre randomised trial on treatment strategies	199	(28)
CLEAR Registry (USA)	early RA disease duration < 2 years African-American		500	(29)
German early RA inception cohort	RA (ACR'87 criteria) age 21-75 years disease duration < 1 year	prospective, multi-centre study referred by GP, rheumatologist, arthritis care units follow-up at least 3 years		(30;31)

specific diagnostic group (7). The percentage of UA patients who developed RA was not mentioned. After 3 years 58% of the UA patients had no symptoms. Twenty-eight percent of the patients in this cohort met the 1987 ACR classification criteria for RA at inclusion.

From the same cohort, 32 patients were described with the diagnosis of non-classified monoarthritis, defined as swelling of a peripheral joint not due to trauma, degenerative

joint diseases or any other specific joint disease (8). Of those 32 patients, 2 (6%) had rheumatoid factor (RF)-positive definite RA after a 3-9 year follow-up. In 29 patients the diagnosis remained "non-classified" arthritis during follow-up.

In the Finnish cohort a group of 47 patients with recent onset RF-negative oligoarthritis was also described (9). After 23 years of follow-up, reclassification of the diagnoses revealed 1 patient with RA, 7 patients with erosions in the hands or feet, 1 patient with systemic lupus erythematosus (SLE), 1 patient with ankylosing spondylitis, 2 patients with "post-traumatic arthritis", 4 patients with osteoarthritis, and 6 patients with reactive arthritis. The other 25 patients presumably still did not fulfil the criteria for a rheumatic disease.

In the UK the Norfolk Arthritis Registry (NOAR) has been following patients with early inflammatory polyarthritis who had been referred by general practitioners (GPs) and local rheumatologists since January 1990, as described by Symmons *et al*. (10). All adults with two or more swollen joints, lasting for at least 4 weeks, could be included. The proportion of UA patients who developed RA was not mentioned in the published data. However, Wiles *et al*. (11) described a study in which the ACR criteria were applied cumulatively, meaning that once a criterion was fulfilled, this criterion was regarded as positive in all subsequent assessments. In this study, 55% of the patients with a symptom duration of less than 2 years satisfied the criteria for RA at inclusion as described above. Sixty-seven percent fulfilled these criteria after one year.

Also from the UK, Quinn *et al*. (12) recently described a cohort of 97 patients with early undifferentiated arthritis of the hands and a disease duration of less than 12 months who were followed for 12 months. RA developed in 14% of the 97 UA patients. Thirty-six percent had persistent synovitis (defined as the presence of 2 or more of the following: joint swelling, joint tenderness or decreased range of motion) after 12 months, whereas 13% were in clinical remission. Only 54% of the patients could be diagnosed with a specific rheumatic disease after a 12-month follow-up.

Initially these patients were included in a cohort of 1877 patients in the Leeds early arthritis clinic of whom 56% had an inflammatory arthritis at inclusion; 50% of these patients had RA and 23% had UA. Patients with UA were classified as having an inflammatory disorder where a specific rheumatic disease could not be diagnosed. It should be noted that patients were eligible for inclusion in the study if they had a history suggestive of inflammatory arthritis, but clinically detectable synovitis was not required. This resulted in the observation that 47% of patients with UA had no synovitis at the time of inclusion.

In Germany Huelsemann *et al*. (13) described a two-year prospective cohort study of patients with "rheumatic symptoms" for less than 1 year's duration who were investigated in an early arthritis clinic in Duesseldorf. The patients were sent to the tertiary referral centre by general practitioners, internists and orthopaedic physicians. Of 320

patients who were investigated, 217 were classified as having inflammatory rheumatic diseases. Of these 217 patients, 117 (54%) could not be diagnosed definitely and were thus considered undifferentiated, and 39 (19%) were diagnosed as having RA. Sixty-eight percent of the patients with UA presented with oligoarticular joint manifestations, while 14% had a monoarticular and 18% had a polyarticular disease (5 or more joints). Follow-up data 4 to 38 months after the initial symptoms were available for 28 patients with UA. Fifteen (54%) of them had a complete remission, 8 patients had unchanged or progressive unclassified disease and 2 (7%) were diagnosed with RA according to the ACR 1987 criteria.

The Austrian early arthritis registry (Austrian Early Arthritis Action, EAA) (14) follows patients with inflammatory arthritis whose symptoms began less than 12 weeks before presentation and who fulfil at least 2 clinical criteria (absence of trauma, joint swelling in at least 1 joint, joint pain in at least 1 joint, morning stiffness > 60 minutes) and at least 1 laboratory criterion (positive RF, ESR > 20 mm/hour, CRP > 5 mg/L, leucocytes > upper limit of normal). Approximately 15% of the patients after 1 year still had no established diagnosis and were classified as having UA. Sixty-five percent of the patients had RA after 1 year, using the ACR 1987 criteria cumulatively as described in the NOAR (15).

In another paper, Machold *et al*. (16) describe 108 patients who had been followed for at least 1 year. At inclusion, 31 patients (29%) had undifferentiated arthritis and 50 patients (46%) were diagnosed with RA. After 1 year, 17 of the UA patients (55%) were diagnosed with RA. The diagnosis of RA was made if patients fulfilled the ACR 1987 criteria, or if clinical examination revealed a polyarthritis of at least 6 weeks duration without evidence of other inflammatory rheumatic diseases. In cases in which the diagnosis could not be ascertained by the rheumatologist, the disease was classified as UA.

Wolfe *et al*. (17) followed 532 patients with undifferentiated arthritis at the Wichita Arthritis Center who at presentation had a symptom duration of at least 2 years. Synovitis was not required if the patient had other clinically suspected characteristics of RA in the history, at physical examination or in laboratory results. 100% were followed up for $\geq$13 months, 93% for $\geq$2 years and 87% for $\geq$ 3 years. 22% of the patients had no joint swelling, and 6% had questionable swelling at the time of inclusion. Fifty-four percent of the cases resolved, while 17% evolved into RA.

A French multi-centre cohort study (18) that includes patients with early arthritis with a maximum duration of 6 months has recently been started. No data on this ESPOIR cohort have been published yet. The study includes RA patients, probable RA patients and patients with a clinical diagnosis of UA that may potentially develop into RA and with at least two inflammatory joints for the past 6 weeks. UA patients with "no potential to develop into RA" are excluded.

In a Dutch study by Jansen *et al*. (19), a group of patients from the Amsterdam early arthritis clinic with peripheral arthritis involving at least 2 joints and a disease duration

of less than 3 years was followed in order to identify variables that could predict an outcome of progressive disease after 1 year. In this study 27% (n=77) of the patients were clinically diagnosed as having UA at inclusion and 72% (n=203) as RA. 42% of the UA patients had oligoarthritis and 58% had polyarthritis. After one year 42% of the patients with UA were categorized as progressive and 58% as mild, using radiographic parameters and the HAQ score as criteria. Thirtyone percent of the progressive UA group (n=10) fulfilled the ACR criteria for RA after one year. From the total UA group, 17% were classified as having RA at 1 year.

The other Dutch cohort is the Leiden Early Arthritis Clinic, which includes patients with any form of arthritis confirmed by a rheumatologist except gout, and a symptom duration of 2 years or less (20). Out of 936 patients at inclusion, 346 (37%) were categorized as having UA and 22% were diagnosed with RA. After one year of follow-up 32% of the UA patients fulfilled the ACR 1987 criteria for RA. The percentage had increased to 40% at 3 years of follow-up (21).

Discussion

We have reviewed inception cohorts with monoarthritis and polyarthritis to evaluate what proportion of patients with UA progress to RA. In the various cohorts these proportions varied considerably. This may be explained by the differences in referral and recruitment procedures, inclusion criteria and, most notably, disease criteria between the various cohorts. The reported proportion of patients with UA who progressed to RA one year after inclusion range between 6% and 55%. However, in the cohorts that required arthritis to be present at inclusion and that defined RAaccording to the ACR 1987 criteria, the proportions range from 17% to 32%.

The part of the Finnish early RA cohort in which only 6% of the patients with UA progressed to RA after a follow-up period of 3 to 9 years (8) probably represents a subgroup of UA, defined as non-classified monoarthritis and RF negative oligoarthritis, and consequently, a small group of patients is concerned (n = 32). Huelsemann *et al*. reported that 7% of his patients with UA developed RA (13). However, at inclusion patients were diagnosed based on clinical expertise and were not classified according to ACR criteria. As only 18% of the UA patients at inclusion had a polyarticular disease, it is possible that a certain proportion of the patients with polyarthritis at inclusion were prematurely diagnosed as having RA. Therefore the proportion of UA patients who progressed to RA might have been underestimated. Also, only 24% of the 117 patients with UA at inclusion were followed. This suggests that these patients represent a subgroup of UA that more often than not has a mild or self-limiting disease course.

Wolfe *et al*. reported that 17% of their UA patients progressed to RA after 3 years (17). The inclusion of patients without synovitis in this cohort could have led to an underestimation of this value however. The same is true for the cohort described by Quinn *et al*. (12). Jansen *et al*. (19) described a cohort of oligo- or polyarthritis patients, and found a 17% progression from UA to RA. In a mixed population of monoand polyarthritis patients, Van Gaalen *et al*. (21) reported that 32% progressed from UA to RA (diagnosis according to the ACR 1987 criteria) within one year. An even higher rate of 55% was described by Machold *et al*. (16). However, in that study not only patients who fulfilled the ACR criteria were diagnosed as having RA, but also patients with polyarthritis for more than 6 weeks without evidence of other inflammatory rheumatic diseases upon investigation. Therefore, the value of 55% could be an overestimation of RA in comparison with other studies that focused only on the ACR criteria for diagnosing RA.

The findings of these cohort studies support the hypothesis that many patients with UA are actually in the first stages of RA. Unpublished observations in the Leiden EAC cohort indicate that patients whose UA evolved into RA within one year have, on average, the same prognosis as patients who presented with RA at baseline, as measured by the rate of joint destruction, disease activity and functional status. Early treatment may moderate the disease progression, possibly to the point that fewer patients develop RA as defined by the ACR 1987 criteria. Ideally, patients with UA who will progress to RA should be identified at presentation in order to receive early aggressive treatment.

Decisions to treat UA patients will depend on the likelihood that a patient will develop RA. When this is high, it is worthwhile to start disease modifying anti-rheumatic drug (DMARD) therapy immediately. Our review shows there is a 17-32% pre-test probability that a patient with UA actually has RA. The question is what tests are available to obtain a substantially higher post-test probability.

A great deal of research has already been carried out to try to identify predictors that could be used for such a test. At present the most promising diagnostic tool appears to be a test for anti-cyclic citrullinated peptide (CCP) autoantibodies. Van Gaalen *et al*. (21) reported that in the Leiden EAC 93% of the patients with UA who were anti- CCP positive fulfilled the ACR 1987 criteria for RA within 3 years. The negative predictive value was 75%. Furthermore, anti-CCP antibody testing was of little value in UA patients who fulfilled none of the ACR 1987 criteria for RA, but had a significant additional value in predicting the progression to RA in UA patients fulfilling one or more of these criteria at presentation. As anti-CCP antibodies can be detected several years before the onset of disease, Holers and Majka (32) proposed a model in which the development of anti-CCP antibodies in genetically predisposed individuals initiates the autoimmune process in a preclinical phase. The presence of anti-CCP antibodies could therefore be used as prediction criteria for the development of RA in patients with UA.

Another more intuitive approach rather than an analytical one is to treat all UA patients with a relatively safe drug regardless of their post-test probability in the event of new predictive tests. This would prevent that "false-negative" patients would not receive aggressive therapy. It is however not (yet) clear how aggressive such a – at the same time safe – therapy could be. It is unclear if such a therapy should be, for example, MTX, corticosteroids or NSAIDs.

Current research is focusing on these treatments and on whether patients with UA will benefit from early treatment with DMARDs to a similar extent as RA patients. In Leiden a doubleblind placebo-controlled randomised trial (Probaat) with 110 patients who fulfill the ACR 1958 criteria for probable RA and with a symptom duration of less then 2 years is now underway. The aim of the study is to determine whether early treatment can prevent progression into RA or even induce remission. The patients are being treated with either placebo or MTX. After one year the medication will be tapered and then stopped.

The study 'Stop Arthritis Very Early' (SAVE) is another placebo-controlled study that has just started and will try to modify the disease course of UA patients whose complaints began less than 16 weeks earlier, with a single injection of methylprednisolone i.m. Subgroup analyses may reveal whether all UA patients need to be treated or if only a proportion of these patients will benefit from early treatment.

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CHAPTER 3

Comparison of long term outcome of patients with rheumatoid arthritis presenting with undifferentiated arthritis or with rheumatoid arthritis: an observational cohort study

J. van Aken*
H. van Dongen*
S. le Cessie
F.C. Breedveld
T.W.J. Huizinga

* both authors contributed equally

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Abstract

Background

The outcome of undifferentiated arthritis (UA) ranges from remission to rheumatoid arthritis (RA) fulfilling the American College of Rheumatology (ACR) classification criteria.

Objectives

To report the outcome of UA after 1-year of follow up and compare the disease course of patients who presented with UA, but evolved into RA within 1 year (UA-RA group), with that of patients who presented with RA fulfilling the ACR criteria (RA-RA group).

Methods

The diagnosis of 330 patients who presented with UA was recorded at 1-year. The UA-RA and RA-RA groups were then followed up for 3 more years. Outcome measurements were radiographic progression, disease activity, and functional capacity.

Results

From 330 patients who were diagnosed UA, 91 had evolved into RA at 1-year; 62 patients had presented with RA. No significant differences were detected between the UA-RA and RA-RA groups in median Sharp/van der Heijde score at baseline, radiographic progression rates, disease activity, and functional capacity. However, significantly more disease modifying antirheumatic drugs were prescribed in the RA-RA group.

Conclusion

The disease outcome of patients who present with UA that evolves into RA within 1 year is the same as that of patients who present with RA as measured by radiographic progression, disease activity, and functional capacity.

Introduction

In various studies of patients with recent onset arthritis, 6–55% of the patients who presented with undifferentiated arthritis (UA) progressed into rheumatoid arthritis (RA) according to the American College of Rheumatology (ACR) classification criteria or according to the rheumatologist (1). Multivariate analysis of characteristics of these patients showed that the presence of antibodies to cyclic citrullinated peptide (anti-CCP), polyarthritis, symmetric arthritis, and erosions on radiographs predict the development of RA. Among these characteristics, anti-CCP has the highest specificity and positive predictive value for RA (2). Thus, either by clinical or by serological data, clinicians will be confronted with patients with UA who are likely to develop RA. The natural course of patients who present with UA and subsequently develop RA is unknown compared with patients who present with RA. In patients with RA many studies have demonstrated the benefit of the early initiation of disease modifying antirheumatic drugs (DMARDs), resulting in less disease activity and reduction of radiographic joint damage (3-5). Patients included in these studies fulfilled the 1987 ACR classification criteria for RA (6). However, the ACR classification criteria were not developed for the purpose of early diagnosis as is illustrated by the fact that a minority of patients with inflammatory polyarthritis fulfilled the ACR criteria at the first visit (7).

Although patients with RA benefit from early aggressive treatment (8), it is unknown whether patients with UA whose disease will evolve into RA will also benefit from such a treatment strategy. Hypothetically, if at presentation the duration of complaints is similar, patients who present with UA might have a disease course that evolves more slowly than that of patients who present with RA ***(figure 1)***. In the worst situation, the "window of opportunity" for early treatment to induce long term disease modification

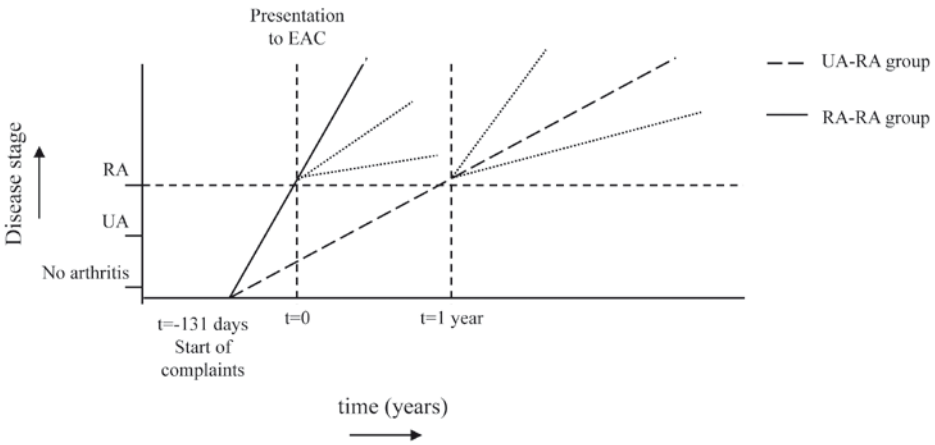

Figure 1. Hypothetical disease course of UA and RA

in the patients who presented with UA has been passed once the diagnosis RA is made, and long term outcome will be comparable or worse than patients who were diagnosed with RA at presentation and treated immediately.

Here, we describe the 1 year diagnosis and outcome of patients who presented with UA, and compare the disease course of the patients with UA who had developed RA within 1 year after presentation (UA-RA group) with that of patients who presented with RA (RA-RA group). Radiographic progression was the major outcome measure; disease activity and functional capacity were minor outcome measures.

Patients and methods

Patients

The Leiden Early Arthritis Clinic (EAC) was started in 1993. General practitioners were asked to refer patients with suspected arthritis as soon as possible. Patients were included in the EAC cohort if a rheumatologist confirmed the suspected arthritis and the symptoms of arthritis did not exceed 2 years; for details of this cohort see Van der Horst-Bruinsma *et al.*(9). Patient history, general physical examination, a painful and swollen joint count, laboratory tests, questionnaires, and radiographic joint scores were performed at inclusion and at the follow up visits. Based on the test results, a diagnosis was recorded at the second visit, 2 weeks later, but could be revised during follow up.

Study design

From the EAC database ***(figure 2)*** patients diagnosed with UA at the second visit were selected and their subsequent diagnosis was recorded at 1 year. UA comprised arthritis of unknown cause and "probable RA" according to the 1958 ACR criteria (10). The group of patients diagnosed with UA who evolved into RA fulfilling the 1987 ACR criteria within 1 year (UA-RA group) was followed up for 4 years. Because these patients had the opportunity to be treated with DMARDs as soon as the diagnosis was made, the long term outcome was compared with that of a group of patients in whom RA was diagnosed at the first and/or second visit and who still had RA after 1 year, and who were treated with DMARDs as soon as RA was diagnosed (RA RA group).

Outcome measurements

Major outcome measurements were radiographic joint damage and calculated radiographic progression rate. Radiographs of hands and feet were taken at inclusion in the EAC and yearly thereafter. Radiographic scoring according to the Sharp/van der Heijde method (11) was performed in random order by an experienced rheumatologist who

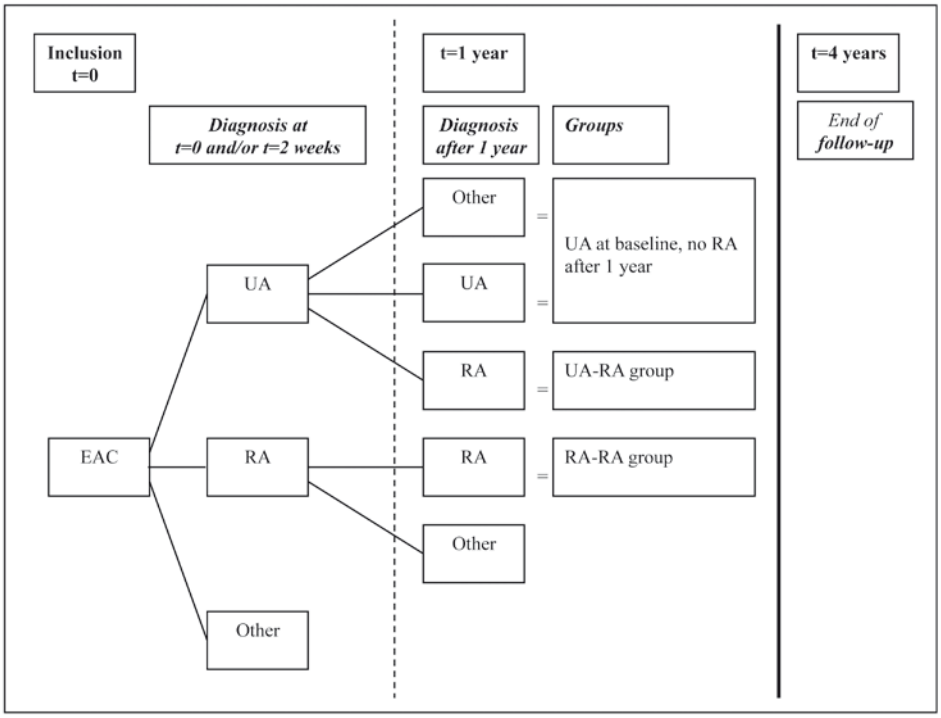

Figure 2. Flow diagram for definition of the groups

was unaware of the clinical data and the study questions. The intraclass correlation coefficient for the assessor's scoring was 0.95, as measured in 62 patients.

Minor outcome measurements were disease activity, functional capacity, erythrocyte sedimentation rate (ESR) in the 1st hour, and C reactive protein (CRP) concentration. Disease activity was calculated by a modified version of the disease activity score (DAS) (8):

$(0.54\times(\sqrt{\text{Ritchie score}})+0.065\times(\text{swollen joint score})+0.33\times\ln \text{ESR}+0.224)$.

Functional capacity was measured by the Health Assessment Questionnaire (HAQ) (12).

Statistical analysis

Differences between patient characteristics were tested with the Mann-Whitney U test or the $\chi 2$ test. For each patient separately, simple linear regression was used to fit the course of the radiographic progression in time. Progression rates were calculated for varying time intervals, assuming that radiographic damage progresses at a constant rate (13;14). The slopes of these regression lines estimate the average increase in Sharp/van der Heijde score per year. Differences between slopes of the groups of interest were tested with the Mann-Whitney U test.

To construct the radiographic progression as depicted in ***figure 3***, the geometric mean of the Sharp/van der Heijde scores was estimated using a linear mixed model, in which account was taken of missing measurements (15). Differences in disease activity and functional capacity were compared with a linear mixed model. All tests were two tailed and p values ≤ 0.05 were considered significant.

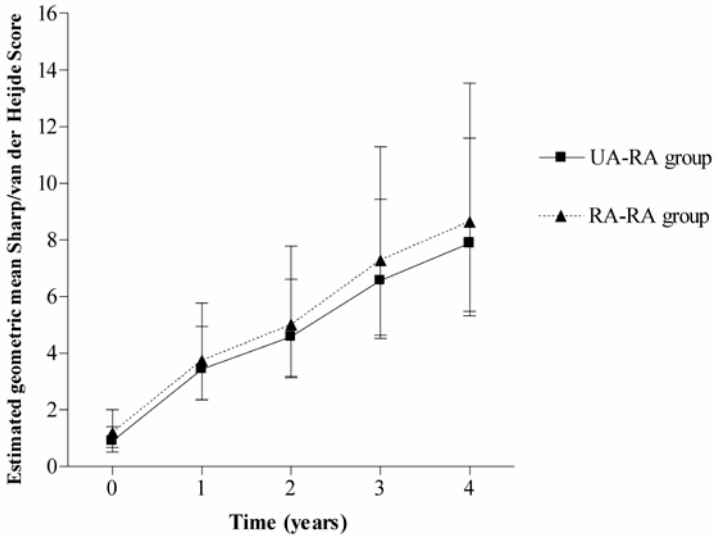

Figure 3. Estimated geometric mean Sharp/van der Heijde Score (95% confidence interval) of the UA-RA and RA-RA groups from baseline to 4 years

Results

Outcome of UA after 1 year

Between 1993 and 1999, 1064 patients were included in the EAC. At presentation, 330 patients were diagnosed with UA. Table 1 shows the diagnosis after 1 year of follow up of patients who had presented with UA and the numbers of patients who had ended follow up and their reasons for doing so. Ninety one patients had developed RA according to the 1987 ACR criteria (UA-RA group).

Patient characteristics at baseline

Table 2 shows the baseline characteristics of the UA-RA group, the RA-RA group, and the group of patients with UA who did not develop RA within 1 year. The UA-RA group differed from the RA-RA group in duration of morning stiffness, number of swollen joints,

Table 1. Diagnosis after 1 year of follow-up of patients who presented with UA and the numbers of patients who had ended EAC follow up at 1 year, and their main reason for doing so.

Diagnosis at 1 year	Patients No (%)	Patients who ended follow-up (n)	Main reason for end of follow-up No (%)
Arthritis of unknown cause	134 (40.6)	76	Remission 57 (75%)
Rheumatoid Arthritis	91 (27.6)	9	Death 3 (33%) Remission 3 (33%) Refusal 1 (11%) Other/unknown 2 (22%)
Probable RA	39 (11.8)	9	Remission 7 (78%)
Osteo-Arthrosis/Arthritis	11 (3.3)	10	End of treatment 7 (70%)
Psoriatic Arthritis	8 (2.4)	2	Remission 2 (100%)
Reactive Arthritis	6 (1.8)	6	Remission 6 (100%)
Crystal Induced Arthritis	4 (1.2)	3	EAC protocol 3 (100%)
Lyme Arthritis	4 (1.2)	2	Remission 2 (100%)
Paraneoplastic Arthritis	3 (0.9)	2	Remission 1 (50%) Life-threatening illness 1 (50%)
Systemic Lupus Erythematosus	2 (0.6)	0	
Palindromic RA	1 (0.3)	1	Move house 1 (100%)
Spondylarthropathy	1 (0.3)	1	EAC protocol 1 (100%)
Unknown cause	6 (1.8)	6	Remission 4 (67%)
Other causes	20 (6.1)	10	Remission 4 (40%)

Table 2. Patient characteristics at baseline of the RA-RA group, the UA-RA group, and patients with UA who did not develop RA within 1 year

Patient characteristics	RA-RA group (n=62)	p-value*	UA-RA group (n=91)	UA at presentation, no RA at 1 year (n=238)
Age (years)	53 (42-72)	0.683	55 (44-65)	46 (35-58)
Female patients	43 (69)	0.492	58 (64)	119 (50)
Duration of symptoms at first visit (days)	130 (61-278)	0.611	131 (73-230)	81 (20-164)
Duration of morning stiffness (min)	90 (45-150)	0.035	60 (18-120)	15 (0-60)
Number of swollen joints	7 (5-8)	<0.001	4 (2-6)	2 (1-3)
Modified DAS score	3.5 (3.1-4.0)	0.012	3.2 (2.7-3.7)	2.3 (1.9-2.8)
Rheumatoid factor positive	44 (71)	0.001	38 (42)	28 (12)
Anti-CCP antibodies	35/59 (59)	0.397	43/83 (52)	16 (8.2)
ESR (mm/1st hour)	38 (20-56)	0.695	31 (19-53)	18 (9-37)
CRP (mg/l)	24 (9-41)	0.414	17 (8-46)	10 (4-26)
HAQ score	1.0 (0.6-1.4)	0.184	0.8 (0.4-1.2)	0.6 (0.1-1.0)
Erosive disease at baseline (hands and feet radiographs)	18/60 (30)	1.000	26/88 (30)	20 (13)
Sharp/van der Heijde score	1 (0-4)	0.281	0 (0-3)	0 (0-0)

*p Value of RA-RA group versus UA-RA group.
Results are shown as number (%) or median (interquartile range).

modified DAS, and percentage of patients who were rheumatoid factor (RF) positive. These differences reflect the ACR criteria for RA.

The patients with UA who did not develop RA within 1 year were significantly different at baseline from both the UA-RA group and the RA-RA group in all characteristics.

Radiographic joint damage

No significant differences were detected between the UA-RA and RA-RA groups for median Sharp/van der Heijde score ***(table 3, figure 3)***. At 4 years 75% of all patients in both the UA-RA group (77%) and the RA-RA group (73%) had erosive disease compared with 30% at baseline.

Table 3. Sharp/van der Heijde scores and radiographic progression rates (increase in score per year) in the UA-RA and RA-RA groups

	UA-RA group (n=91)		**RA-RA group (n=62)**	
	n	**Median (IQR)**	**n**	**Median (IQR)**
Sharp/van der Heijde score				
Baseline	88	0 (0-3)	60	1 (0-4)
1 year	79	4 (0-13)	56	5 (0-18)
2 years	71	8 (0-17)	48	5 (1-27)
3 years	66	10 (2-20)	45	11 (2-26)
4 years	53	9 (2-30)	41	15 (4-27)
Radiographic progression rate				
Slope 0-1 year*		2 (0-8)		2 (0-10)
Slope 0-2 years*		3 (0-8)		1 (0-8)
Slope 0-4 years*		2 (0-7)		2 (0-4)
Slope 1-4 years*		2 (0-8)		1 (-1-3)
Slope 2-4 years*		2 (-1-8)		2 (0-5)

*p>0.05 in the UA-RA group versus the RA-RA group.
n is the number of patients with available data.

Disease activity and functional capacity

Figures 4A-D show the mean DAS, HAQ, ESR, and CRP of the two groups. During follow up no significant differences were seen.

DMARDs

Table 4 shows the use of DMARDs. At all time points significantly fewer DMARDs were prescribed in the UA-RA group than in the RA-RA group. Frequently prescribed DMARDs were hydroxychloroquine, sulphasalazine, and methotrexate or a combination of these.

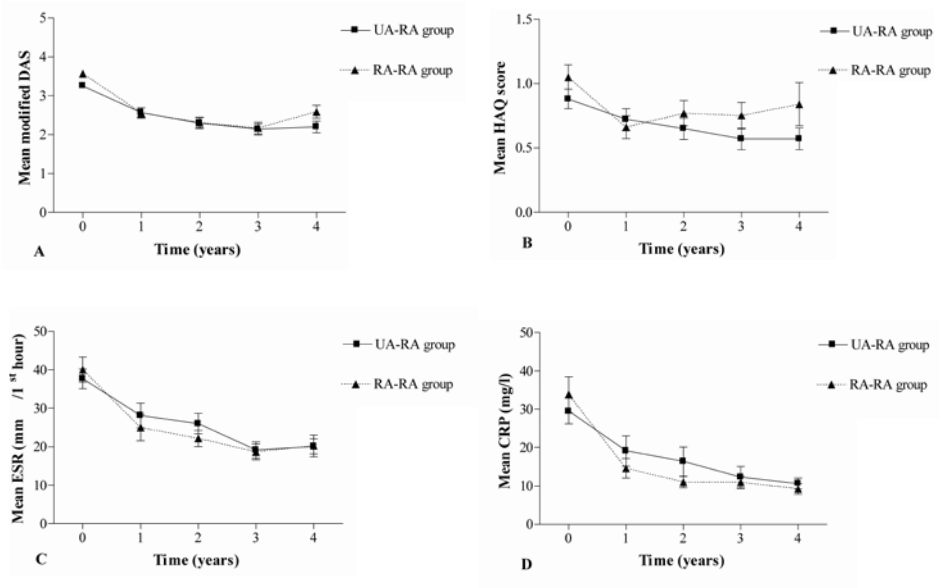

Figure 4. Secondary outcome values of the UA-RA and RA-RA groups from baseline to 4 years. (A) Modified Disease Activity Score; (B) Health Assessment Questionnaire score; (C) Erythrocyte Sedimentation Rate in mm/1st h; (D) C reactive protein concentration. Mean values (SEM).

Table 4. Number (%) of patients receiving prescribed DMARDs at 3 months, 1, 2, 3 and 4 years in the UA-RA and RA-RA groups

Time points	UA-RA group (n=91)	RA-RA group (n=62)	p Value
3 months	42 (46)	62 (100)	<0.001
1 year	62 (68)	58 (94)	<0.001
2 years	63 (69)	56 (90)	<0.001
3 years	60 (66)	49 (79)	0.04
4 years	52 (57)	48 (77)	0.003

Prednisolone was also prescribed as single drug or combination therapy. Gold, azathioprine, and ciclosporin were prescribed in a minority of patients, and biological agents were not prescribed at all.

In the UA-RA group, 16/91 patients never received DMARDs during the follow up period of 4 years. Of these 16 patients, 11 were RF negative and in 10 patients erosive disease was seen during follow up.

Dropouts

Follow up of 3 - 4 years was available for 124 patients. Twenty nine patients were lost to follow up (dropouts). In the UA-RA group five patients were in remission, two refused follow up, five had died, one discontinued because of a concomitant illness, and in six

patients the cause was unclear. In the RA-RA group four patients were in remission, two refused follow up, one had died, one discontinued because of a concomitant illness, and in two patients the cause remained unclear. ***Table 5*** compares the baseline characteristics of the dropouts with those of patients with 4 year follow up (completers). Only CRP in the UA-RA group was significantly higher in the dropout group than for the completers

Table 5. Baseline characteristics of dropouts (before or at 3 years of follow-up) versus completers

	UA-RA group			RA-RA group		
Patient characteristics	**Dropouts (n=19)**	**Completers (n=72)**	**p Value**	**Dropouts (n=10)**	**Completers (n=52)**	**p Value**
Age (years)	64 (46-75)	55 (44-63)	0.099	57(36-85)	53 (46-69)	0.745
Female patients	57	65	0.598	60	71	0.479
Duration of symptoms at first visit (days)	102 (61-209)	144 (78-250)	0.381	159 (63-275)	126 (59-290)	0.851
Duration of morning stiffness (min)	60 (30-180)	60 (15-120)	0.341	75 (41-135)	90 (45-180)	0.875
Number of swollen joints	5 (3-8)	4 (2-5)	0.087	7 (4-8)	7 (5-8)	0.658
Modified DAS score	3.3 (2.8-4.2)	3.1 (2.6-3.6)	0.218	3.5 (2.9-4.7)	3.5 (3.1-4.0)	0.626
RF- positive	26	47	0.127	60	73	0.457
anti-CCP antibodies	42	55	0.435	50	61	0.725
ESR (mm/1st hour)	37 (25-65)	30 (18-50)	0.091	46 (25-61)	35 (19-57)	0.410
CRP (mg/l)	57 (13-74)	11 (7-32)	0.002	19 (9-44)	27 (9-42)	0.984
HAQ score	1.0 (0.12-1.37)	0.75 (0.5-1.12)	0.852	1.31 (0.65-1.75)	1.0 (0.5-1.37)	0.382
Erosive disease (hands and feet radiographs)	33	29	0.695	44	28	0.309

Results are shown as number or median (interquartile range)

Discussion

Here, the hypothesis was tested whether patients with UA who developed RA within 1 year have a different disease course than those who presented to the Leiden EAC with RA fulfilling the ACR criteria (6). Of the 330 patients who initially presented with UA, 91 (28%) had developed RA within 1 year. Although the patients with UA might hypothetically have evolved more slowly towards RA, joint damage, disease activity, and functional capacity were the same in both groups over a follow up period of 4 years.

UA is a commonly encountered problem in daily practice. In one third of patients with recent onset arthritis it is not possible to come to a definite diagnosis at presentation. Overall, 63% of the patients at the Leiden EAC had a self limiting disease course and 28% progressed from UA to RA. Other studies show that 6 - 55% of patients with UA will even-

tually evolve into RA (1). These percentages are dependent on the different inclusion criteria, recruitment procedures, and disease criteria. For example, low percentages are found in RF negative patients with monarthritis (16), and higher percentages are found in inception cohorts where patients with polyarthritis are diagnosed with RA, regardless of fulfilment of the ACR criteria (17).

Despite the fact that many studies have tried to describe the natural history of UA, the long term outcome of the subgroup of patients with UA who develop RA has never been described. As can be expected, the UA-RA and RA-RA groups differed at presentation in duration of morning stiffness, number of swollen joints, modified DAS, and percentage of RF positive patients, because these variables are directly or indirectly part of the 1987 ACR classification criteria for RA. The presence of anti-CCP was equal in both groups and is similar to previous studies (18). The group of patients with UA who did not develop RA within 1 year differed significantly in all patient characteristics at baseline from the other two groups. This is a very heterogeneous group considering the diagnosis after 1 year, but all these patients were diagnosed with UA at baseline.

Median symptom duration is about the same in both groups. However, given the large variation in symptom duration some misclassification of patients might have occurred. Theoretically, patients with UA who presented with short symptom duration might have belonged to the RA-RA group, and patients with RA with very long symptom duration might have had the chance of being included in the UA-RA group. However, analysis of subgroups with different intervals of symptom duration did not show an effect on joint damage.

In the dropout analysis, only CRP in the UA-RA group was significantly higher in the dropout group than for the completers. These data argue against the possibility that the UA-RA group equals the RA-RA group because the dropouts in the UA-RA group were the patients who presented with a milder disease, and only the patients with more aggressive disease completed the follow up.

At all time points, the UA-RA group received fewer DMARDs than the RA-RA group. In this observational study, this might reflect a historical development in DMARD prescription. It also might be a confounding by indication, as clinicians are inclined to prescribe DMARDs to patients who they expect to have a more destructive disease course. Then, patients in the RA-RA group might have a more aggressive disease course that is modified by DMARDs. However, the power to detect an effect of the presence or absence of DMARD use on radiographic progression was too low given the small number of patients with RA who did not use DMARDs (n=16). In the UA-RA group, 16/91 patients did not receive DMARDs during the 4 year follow up. Although 11 of these patients were RF negative, 10 had erosive disease. However, with a median radiographic progression of one point a year, this subgroup had less progressive disease than the group treated

with DMARDs. A randomised clinical trial with DMARDs in patients with UA would be the most appropriate study design to overcome this issue.

The overall outcome of UA poses challenging treatment goals. Patients with UA who will go into remission will need treatment aimed at reducing symptoms, whereas patients prone to progress to RA will need treatment to improve their long term outcome as well. Furthermore, there is a need for new criteria to identify patients with UA that will evolve into RA, as the current ACR criteria for RA are not suitable for this purpose. This study implies that the long term outcome of patients who present with UA which evolves into RA is the same as that of patients who present with RA. New serological tests combined with clinical judgment will help to determine which patients with UA are most likely to develop RA. Randomised clinical trials in such patients with UA are currently under way to test whether this group of patients may benefit from very early DMARD treatment.

Acknowledgements

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CHAPTER 4

Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis. A double-blind, randomized, placebo-controlled trial

H. van Dongen*
J. van Aken*
L.R. Lard
K.Visser
H.K. Ronday
H.M.J. Hulsmans
I. Speyer
M.L. Westedt
A.J. Peeters
C.F. Allaart
R.E.M. Toes
F.C. Breedveld
T.W.J. Huizinga

* both authors contributed equally

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Abstract

Objective

To determine whether patients with undifferentiated arthritis (UA; inflammatory, non-traumatic arthritis that cannot be diagnosed using current classification criteria) benefit from treatment with methotrexate (MTX).

Methods

The PRObable rheumatoid arthritis: Methotrexate versus Placebo Treatment (PROMPT) study was a double-blind, placebo-controlled, randomized, multicenter trial involving 110 patients with UA who fulfilled the American College of Rheumatology (ACR) 1958 criteria for probable RA. Treatment started with MTX (15 mg/week) or placebo tablets, and every 3 months the dosage was increased if the Disease Activity Score was >2.4. After 12 months, the study medication was tapered and discontinued. Patients were followed up for 30 months. When a patient fulfilled the ACR criteria for RA (primary end point), the study medication was changed to MTX. Joint damage was scored on radiographs of the hands and feet.

Results

In 22 of the 55 patients (40%) in the MTX group, UA progressed to RA compared with 29 of 55 patients (53%) in the placebo group. However, in the MTX group, patients fulfilled the ACR criteria for RA at a later time point than in the placebo group ($P = 0.04$), and fewer patients showed radiographic progression over 18 months ($P = 0.046$).

Conclusion

This study provides evidence for the efficacy of MTX treatment in postponing the diagnosis of RA, as defined by the ACR 1987 criteria, and retarding radiographic joint damage in UA patients.

Introduction

For a number of autoimmune diseases, such as diabetes mellitus, it has been suggested that a critical period exists during which intervention may reverse the disease process (1). For rheumatoid arthritis (RA), such a window of opportunity may also exist, because laboratory abnormalities, such as antibodies against cyclic citrullinated peptide (anti-CCP), can occur years before disease onset (2). In previous studies of patients with undifferentiated arthritis (UA), defined as an inflammatory arthritis in which no definitive diagnosis can be made (3), it was observed that the presence of anti-CCP, with an odds ratio of 38, is an important predictor of the development of RA (4). Of all the patients who present with UA, depending on the study population, 6-55% develop RA within 1 year (5).

Current treatment of UA patients mainly consists of nonsteroidal antiinflammatory drugs (NSAIDs), and treatment with disease-modifying antirheumatic drugs (DMARDs) is not initiated until the disease has progressed to RA (3). Once patients fulfill the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 criteria for RA (6), early initiation of DMARD treatment results in less disease activity, reduction of radiographic joint damage, and maintenance of function, as compared with delayed treatment (7-9). Consequently, it is hypothesized that DMARD treatment that is started as early as possible in UA patients may alter disease progression and may prevent the development of RA.

Therefore, we designed a double-blind, placebo-controlled, randomized clinical trial to compare 2 treatment strategies. The immediate treatment strategy consisted of methotrexate (MTX) for a course of 1 year followed by tapering the amount of treatment if UA had not evolved into RA (as defined by fulfillment of the ACR classification criteria). The control group received conventional treatment with NSAIDs, and MTX therapy was initiated only if the patients fulfilled the ACR criteria for RA (the primary end point). The primary outcomes of the PRObable RA: Methotrexate versus Placebo Treatment (PROMPT) study were diagnosis at the end of the study and progression of radiographic joint damage.

Patients and methods

Study setting and design

The PROMPT study was a prospective double-blind, randomized, placebo-controlled, multicenter trial involving 110 patients. Randomization was performed by the pharmacist. The study was conducted between March 2001 and January 2006 in 4 hospitals in Leiden, The Hague, and Delft, The Netherlands. The study was approved by the medical ethics committee of the participating hospitals. Patients started with 6 tablets, each

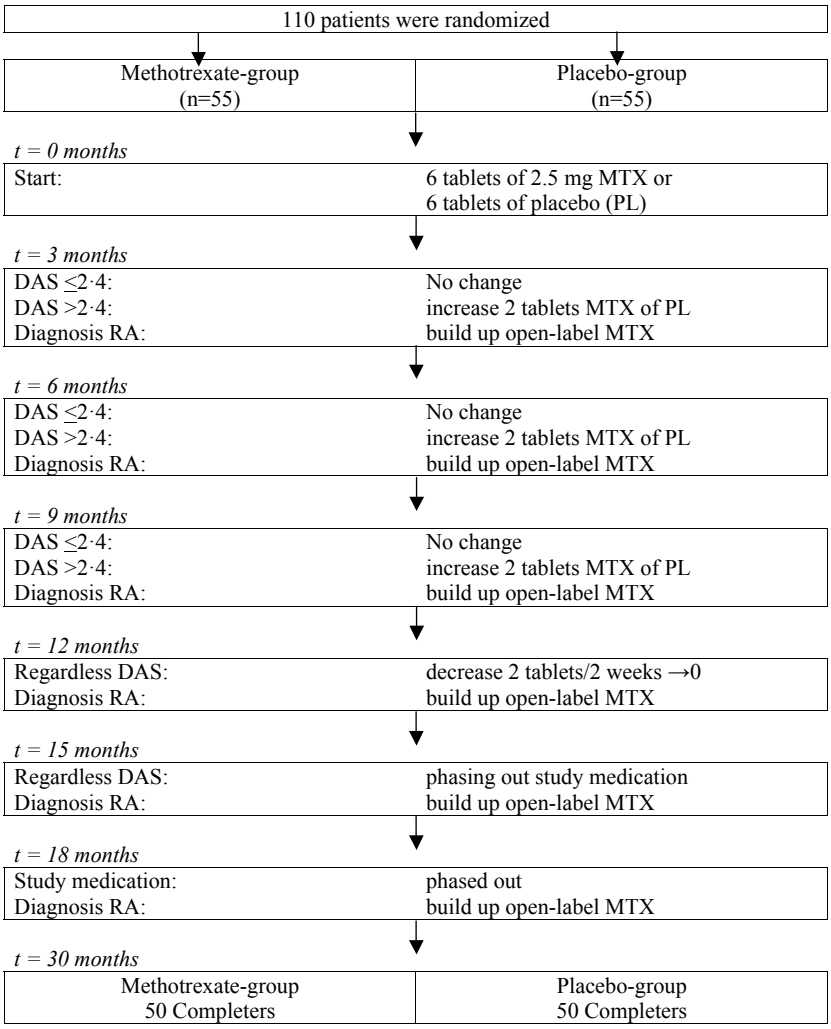


Figure 1. Overview of treatment strategy. Patients received open-label methotrexate (MTX) if rheumatoid arthritis (RA; n=49) or psoriatic arthritis with erosive disease (n=2) was diagnosed. PL = placebo; DAS = Disease Activity Score.

containing either 2.5 mg MTX or placebo. Every 3 months, the medication was increased by 2 tablets if the Disease Activity Score (DAS) was >2.4 (10), to a maximum of 12 tablets or 30 mg of MTX ***(Figure 1)***. The DAS was calculated using a tender joint count (Ritchie Articular Index; RAI), the erythrocyte sedimentation rate (ESR), and a visual analog scale (VAS) for general health status, according to the following formula:

$$DAS = 0.54\times\sqrt{(RAI)} + 0.065\times(\text{swollen joint count}) + 0.33\times\ln(ESR) + 0.0072\times(\text{VAS general health})$$

Trained research nurses calculated the tender and swollen joint scores. To minimize the side effects of MTX, all patients, including those in the placebo group, received folic acid daily (1 mg) or weekly (5 mg). In both groups, patients were allowed to take NSAIDs, but no other immunosuppressive therapies, including steroids, were allowed. In cases of side effects that might be related to MTX, the treatment was adjusted.

If a patient reached the primary end point during followup, defined as fulfilling the ACR 1987 RA classification criteria, it was considered unethical to continue with study medication (possibly placebo), and the treatment was initially continued by building up open-label MTX to the same amount as in the study medication scheme. After 12 months, the study medication was decreased by 2 tablets every 4 weeks until it reached a level of 0 in the patients who did not reach the primary end point. At study inclusion and at 3, 6, 9, 12, and 18 months thereafter, a tender and swollen joint count, a Health Assessment Questionnaire, and a VAS for general health were obtained (11;12). Every 6 months, radiographs of hands and feet were obtained. At 30 months, the diagnosis was recorded.

Participants

Eligible patients attended the rheumatology outpatient clinic of the participating hospitals, had symptoms of arthritis that did not exceed 2 years in duration, were 18 years of age or older, and were diagnosed as having UA (i.e., did not fulfill classification criteria for any rheumatologic disorder). Given the lack of criteria for UA, patients had to fulfill the ACR 1958 criteria for probable RA (13). One patient with psoriasis was also included because the small joints of the hands and feet were involved. Exclusion criteria were RA (according to the ACR 1987 criteria), impaired kidney or liver function, alcoholism, bone marrow insufficiency, pregnancy or the desire to become pregnant within 21 months from inclusion in the study, and DMARD use in the past. All patients provided written informed consent.

Outcome

The diagnosis at the end of the study and the radiographic progression were prespecified primary outcomes. After 30 months, a diagnosis of RA, UA, remission, or other was recorded. Remission was defined as no clinical symptoms of arthritis according to the patient's rheumatologists and no DMARD use in the preceding year.

Radiographic damage was graded by 2 experienced readers (JvA and HvD) using the Sharp/van der Heijde scoring method (SHS), with the radiographs in chronological order and patient identity masked (14;15). The interobserver intraclass correlation coefficient (ICC) was 0.898. The intraobserver ICCs for both readers were 0.990 and 0.993, as measured in 20 patients. The smallest detectable change (SDC) was 3.02 (16). Prespecified

secondary outcomes were changes in disease activity represented by the ESR and the DAS.

For a post hoc analysis of the outcomes according to anti-CCP status, the presence of anti-CCP was measured at the end of the study in baseline serum samples from all patients, before decoding the treatment arms.

Statistical analysis

Using historical data from the Leiden Early Arthritis Clinic (EAC), 40% of the patients with UA were expected to develop RA within 1 year (4). It was estimated that immediate treatment with MTX would result in <20% of the patients with UA developing RA. A sample size of 46 patients per treatment group was required in order to attain a power of 80% to detect a significant difference between groups with a *P* value of 0.05. To allow for dropouts, 110 patients were included in the study.

Demographic and baseline characteristics, changes in the DAS and ESR, and radiographic progression in the 2 treatment groups were compared using the chi-square test, Student's 2-tailed t-test, or the Mann-Whitney U test. Because MTX reduces radiographic joint damage in RA and the SHS method does not allow for healing, radiographic progression was tested 1-sided (15;17). Differences in the development of RA during the study were determined using a Kaplan-Meier curve with a log rank test. The Cox proportional hazards model yielded the hazard ratios and 95% confidence intervals. *P* values less than 0.05 were considered statistically significant.

Results

Characteristics of the study patients

The demographic and clinical characteristics of the patients at baseline are shown in ***Table 1***. To allow for assessment of the external validity of this trial, the baseline characteristics of the total group of patients in the PROMPT study were compared with a control group of UA patients who were included in the Leiden EAC study between 1993 and 1999 (3). In the PROMPT study group, the duration of symptoms was longer and the proportion of patients who were rheumatoid factor (RF)-positive was higher than in the EAC group. The ESR and C-reactive protein (CRP) levels were lower in the PROMPT group than in the EAC group.

Diagnosis at the end of the study

Figure 1 shows the randomization and treatment strategy. After 30 months, in 22 of 55 of the patients in the MTX group (40%) versus 29 of 55 in the placebo group (53%) UA had eventually progressed to RA. However, in the placebo group, all patients whose

Table 1. Demographic and clinical characteristics at baseline of members of the PROMPT study and of the UA patients in the Leiden EAC study*

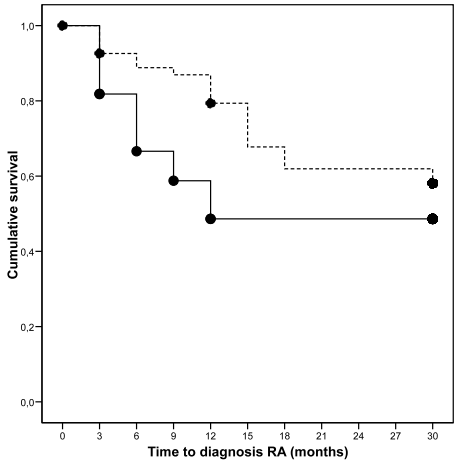
	MTX group n=55	Placebo group n=55	EAC group n=330
Age, years	51 (42-60)	51 (42-56)	48 (36-61)
Female, no. (%)	35 (64)	38 (69)	177 (54)†
Duration of symptoms at first visit, days	312 (195-507)	263 (169-432)	92 (31-186) †
Duration of morning stiffness, minutes	30 (10-60)	30 (10-60)	30 (0-60)
No. of swollen joints	3 (2-5)	2 (2-4)	2 (1-4) †
3-variable DAS	2.7 (2.1-3.1)	2.4 (2.0-3.0)	2.5 (2.0-3.1)
4-variable DAS	2.7 (2.2-2.7)	2.5 (2.0-3.0)	
RF positive, no. (%)	20 (36)	19 (35)	66 (21) †
Anti-CCP positive, no. (%)	12 (22)	15 (27)	55 (20)
ESR, mm/hour	12 (5-24)	11 (5-25)	22 (10-40) †
CRP, mg/liter	5 (3-11)	5 (3-9)	11 (5-31) †
HAQ score	0.75 (0.38-1.13)	0.75 (0.25-1.13)	0.62 (0.25-1.12)
Patients with erosive disease, no. (%)	2 (4)	3 (6)	37 (16) †
Sharp/van der Heijde score	0.5 (0-2.5)	1 (0-3.0)	0 (0-1.0) †

* Except where indicated otherwise, values are the median (interquartile range). PROMPT = PRObable rheumatoid arthritis: Methotrexate versus Placebo Treatment; UA = undifferentiated arthritis; EAC = Early Arthritis Clinic; MTX = methotrexate; DAS = Disease Activity Score; RF = rheumatoid factor; anti-CCP = anti-citrullinated peptide antibody; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; HAQ = Health Assessment Questionnaire.
†$P \leq 0.05$ versus the PROMPT group.

disease had progressed to RA fulfilled the ACR criteria within 1 year, versus only one-half of the RA patients in the MTX group ($P = 0.04$) ***(Figure 2A)***. The other half of the RA patients in the MTX group reached the diagnosis during or after tapering of the study medication. After 30 months, a similar number of patients achieved remission in both treatment groups: 15 in the MTX group and 13 in the placebo group ***(Table2)***.

Radiographic progression

The distribution of the radiographic progression over 18 months is shown in ***Figure 3A***. In both groups, 51 patients had completed radiographic followup. After 18 months, the majority of patients had no radiographic progression at all: 73% in the placebo group and 88% in the MTX group. However, 6 patients in the MTX group versus 14 patients in the placebo group showed radiographic progression above the SDC ($P = 0.046$). Individual progression measured only in patients with erosions was significantly lower in the MTX group versus the placebo group ($P = 0.035$).



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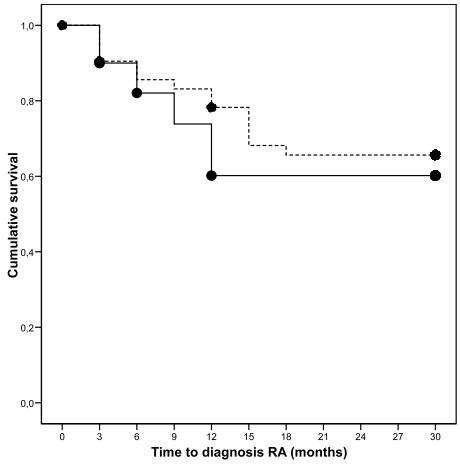

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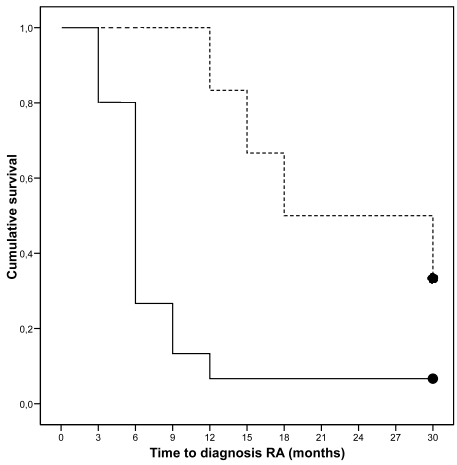

B

Figure 2. Kaplan-Meier survival analysis for the diagnosis of rheumatoid arthritis (RA). The methotrexate (MTX) group is indicated by the broken line, the placebo group is indicated by the solid line, and drop-outs are indicated by circles. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) indicate the risk of developing RA during the study in the placebo group versus the MTX group. (A) Total group (n=110) (HR 1.7 [95% CI 0.99-3.01], *P*=0.04). (B) Members of the subgroup positive for antibodies against cyclic citrullinated peptide (anti-CCP) (n=27) (HR 4.9 [95% CI 1.88-12.79], *P*<0·001). (C) Members of the subgroup negative for anti-CCP (n=83) (HR 1.3 [95% CI 0.61-2.63], *P*=0.51).

Subgroup analysis

In the anti-CCP-positive subgroup treated with placebo, UA in 14 of the 15 patients (93%) progressed to RA and did so at an earlier time point than that in 8 of the 12 patients (67%) in the MTX group ($P < 0.001$) ***(Table 2 and Figure 2B)***. In contrast, in the anti-CCP-negative subgroup no differences in outcome at 30 months were seen ***(Figure***

Table 2. Diagnosis at 30 months, by subgroup*

Group (n)	RA	UA	UA in remission	Other	Lost to follow up
Total					
MTX (55)	22	10	15	3 (2 osteoarthritis, 1 autoimmune hepatitis)	5
Placebo (55)	29	4	13	4 (3 osteoarthritis, 1 diabetic arthropathy)	5
Anti-CCP-positive					
MTX (12)	8	2	2	0	0
Placebo (15)	14	0	1	0	0
Anti-CCP-negative					
MTX (43)	14	8	13	3 (2 osteoarthritis, 1 autoimmune hepatitis)	5
Placebo (40)	15	4	12	4 (3 osteoarthritis, 1 diabetic arthropathy)	5

* RA = rheumatoid arthritis; UA = undifferentiated arthritis; MTX = methotrexate; anti-CCP= anti-cyclic citrullinated peptide antibody

2C). Similar effects on radiographic progression were observed ***(Figures 3B and C)***. In the anti-CCP-negative group, no MTX effect could be detected, whereas in the anti-CCP-positive group, the progression was slowed down significantly ($P = 0.03$).

Subgroup analysis for the presence of RF showed considerable overlap with the presence of anti-CCP: 26 of 39 RF-positive patients (67%) were anti-CCP positive, whereas 60 of 61 RF-negative patients (98%) were anti-CCP negative. In the placebo group, UA in 13 of the 19 RF-positive patients (68%) progressed to RA and did so at an earlier time point than did 11 of the 20 RF-positive patients (55%) in the MTX group ($P = 0.036$). In contrast, in the RF-negative subgroup, no differences in outcome at 30 months were seen ($P = 0.403$). With regard to radiographic progression, RF-positive patients in the placebo group showed a trend for more radiographic progression than those in the MTX group (data not shown).

Subgroup analysis of the development of RA over time for autoantibody-positive (anti-CCP-positive or RF-positive) patients weakened the significance as compared with analysis of the anti-CCP-positive patients alone ($P = 0.024$). Of the 27 anti-CCP-positive patients, only 1 was RF negative. This patient received placebo and was diagnosed as having RA 6 months after study inclusion. The outcome of the 26 patients who were anti-CCP-positive and RF-positive is shown in ***Table 2***.

Cross-sectional followup

At the time of submission of this article, further followup data were not available for all patients due to different followup periods. However, we can report on 5 of 27 patients in the anti-CCP-positive subgroup (18%) who did not develop RA after 30 months ***(Table 2)***. The 3 patients who were in remission at 30 months (1 in the placebo group and 2 in the MTX group) were still in remission without DMARD treatment 4 years after study inclusion. However, the 2 patients with persistent UA (both from the MTX group) restarted MTX treatment after 30 months, 1 because of recurrent symptoms and, ulti-

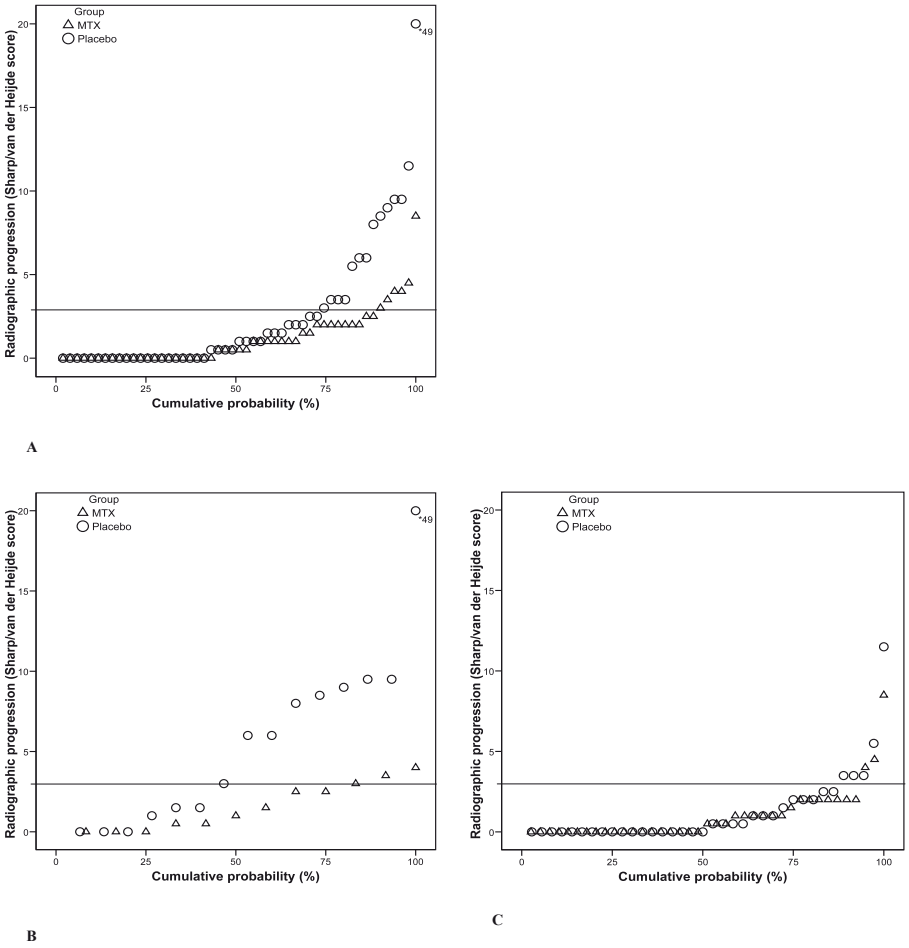

Figure 3. Occurrence of radiographic progression. The presence or absence of radiographic progression (Sharp/van der Heijde score) at 18 months in 102 patients who completed radiographic followup was calculated. Each symbol represents 1 patient. Horizontal lines represent the smallest detectable difference (indicating radiographic progression). A, Total group (n = 102). B, Patients positive for antibodies against cyclic citrullinated peptide (anti-CCP) (n = 27). C, Patients negative for anti-CCP (n = 75). For methotrexate (MTX)-treated versus placebo-treated patients, $P = 0.15$ in the total group, $P = 0.03$ in the anti-CCP-positive patients, and $P = 0.46$ in anti-CCP-negative patients.

mately, arthritis shortly after withdrawal of study medication and 1 because of recurrent arthritis after a 6-month remission period. The patients continued to receive MTX at the time of submission of this article. As a result, although only 1 fulfilled the ACR criteria for RA, it can be argued that they both have RA, given the recurrent arthritis symptoms that require DMARDs. Taking these data into account, 10 of 12 anti-CCP-positive patients (83%) in the MTX group eventually developed RA and 2 of 12 patients (17%) achieved sustained remission, emphasizing that MTX postponed, but did not prevent, RA.

Disease activity

After 3 months, the mean decrease in DAS (from 2.7 to 2.3) and ESR (from 17 mm/hour to 13 mm/hour) values in the MTX group differed significantly from the mean change in DAS (from 2.5 to 2.5) and ESR (from 15 mm/hour to 16 mm/hour) in the placebo group ($P = 0.01$ and $P = 0.02$, respectively). However, subgroup analysis showed that in the anti-CCP-positive patients, the mean DAS decreased (from 2.8 to 1.9) in the MTX group and increased in the placebo group (from 2.6 to 3.1) ($P < 0.001$). However, in the anti-CCP-negative patients, the mean DAS decreased irrespective of treatment group (from 2.7 to 2.4 versus from 2.5 to 2.3 in the MTX and placebo groups, respectively; P = 0.62).

After 12 months, study medication was decreased regardless of the DAS. In the MTX group, 8 of 40 patients who still received study medication had a DAS >2.4 at 12 months and started phasing out the study medication; the UA in 2 of these 8 patients later progressed to RA. Of the 32 patients with a DAS ≤2.4, the UA in 9 of them progressed to RA. Of the anti-CCP-positive patients in the MTX group, only 1 of the 10 who still received study medication at 12 months had a DAS >2.4 and later developed RA. Of the 9 patients with a DAS ≤2.4, 5 developed RA, 2 had UA without fulfilling the ACR criteria for RA at 30 months, and 2 went into remission.

Toxicity

Adverse events were recorded during the first 18 months, the intervention period of the study. Of the patients who were taking study medication, 26 of 55 patients (47%) in the MTX group and 18 of 55 patients (33%) in the placebo group experienced ≥1 (serious) adverse event ($P = 0.173$). While taking the study medication, a total of 44 adverse events and 5 serious adverse events occurred in the MTX group versus a total of 25 adverse events and 4 serious adverse events in the placebo group ***(Table 3)***.

When patients fulfilled the ACR 1987 RA classification criteria during followup, they were switched to open-label MTX. If MTX produced undesirable adverse events or was ineffective, other DMARDs were prescribed. The 20 patients who developed RA in the former MTX group in the first 18 months were subsequently treated with MTX (19 patients), sulfasalazine (1 patient), hydroxychloroquine (3 patients), and/or leflunomide (1 patient). Adverse events (12 adverse events and 3 serious adverse events) were reported in 7 of 20 patients while they were treated with these DMARDs. In the former placebo group, the 29 patients who developed RA in the first 18 months were treated with MTX (29 patients), sulfasalazine (2 patients), hydroxychloroquine (5 patients), leflunomide (1 patient), infliximab (2 patients), etanercept (1 patient), and/or adalimumab (2 patients). Adverse events (39 adverse events and 3 serious adverse events) were reported in 18 of 29 patients while they were treated with these DMARDs.

Table 3. Adverse events and serious adverse events during the use of the study medication and during the use of DMARDs after RA diagnosis, by treatment group*

	During use of study medication		During use of DMARDs after diagnosis RA	
	MTX	**Placebo**	**Former MTX**	**Former Placebo**
All adverse events				
Gastrointestinal	11	6	1	12
Dermal / mucosal	9	7	2	5
Neurologic	3	5	5	4
Cardiologic	3	-	-	2
Pulmonary	3	1	3	1
Haematologic	1	1	-	-
Ophtalmologic	3	-	1	2
Elevated serum liver enzyme levels	6	1	-	4
Other				
Tiredness	1	1	-	2
Giant cell tumor	1	-	-	-
Rhinitis	1	-	-	-
Not feeling well	2	-	-	1
Fracture	-	1	-	-
Hair loss	-	1	-	3
Synovectomy	-	1	-	1
Arthroplastic surgery	-	-	-	1
Weight gain and oedema	-	-	-	1
Total	44	25	12	39
Serious adverse events				
Necessitating discontinuation of study medication				
Gastrointestinal	1	1	NA	NA
Erythema annulare centrifugum	1	-	NA	NA
General unwellness	-	1	NA	NA
Dyspnea, insomnia, weight gain	-	1	NA	NA
Necessitating hospital admission				
Pancreatitis	1	-	-	-
Knee replacement surgery	1	-	-	-
Erosive arthritis	1	-	2	3
Meningitis	-	1	-	-
Venous thrombosis	-	-	1	-
Total	5	4	3	3

* Values are the number of events. DMARDs = disease-modifying antirheumatic drugs; RA = rheumatoid arthritis; MTX = methotrexate; NA = not available.

Discussion

This study indicates that MTX treatment of patients with UA can postpone progression to RA, as defined by fulfillment of the ACR 1987 criteria, and can retard radiographic joint damage. However, the results do not suggest that a 1-year course of MTX treatment can prevent the development of RA from UA. Although these findings must be confirmed in future trials, the PROMPT study provided the first evidence of the efficacy of MTX treatment in UA patients.

Data from the current study showed that initiation of MTX treatment in UA patients in the stage before they fulfilled the ACR criteria for RA resulted in postponement of the diagnosis of RA. During the first year, the incidence of RA was lower in the MTX group than in the placebo group, but after 12 months, the opposite was seen. Furthermore, the results suggest that MTX did not induce more remission, but prolonged the period of persistent UA. Interestingly, the benefit of this effect seemed to be the retardation of radiographic progression. The majority (62%) of the 29 RA patients in the placebo group had already fulfilled the ACR criteria and started taking open-label MTX within 6 months. Nevertheless, the results still show a more favorable outcome in the group that immediately received MTX after study inclusion.

This is the first double-blind, randomized, placebo-controlled trial that addresses early DMARD treatment in patients with UA before they fulfill the ACR criteria for established RA. Although these criteria are currently under debate for use in clinical practice, for research purposes they are regarded as the standard for objectively describing the RA phenotype and have been widely used as inclusion criteria for trials. Moreover, results from many randomized controlled trials have shown that in patients who fulfill the ACR criteria for RA, DMARDs improve the outcome (18;19). Thus, for ethical reasons, a placebo-controlled trial in UA patients could not be extended once the patients fulfilled the criteria. Therefore, the primary outcome of the study, the diagnosis of RA as defined by fulfillment of the ACR 1987 classification criteria, seems a reasonable end point.

Patients with UA included in this study are not completely representative of the average UA patient. This was illustrated by comparing the data from the patients in the PROMPT study with controls from the Leiden EAC. Longer symptom duration and lower ESR and CRP levels were seen in the patients in the PROMPT study. This indicates that the UA patients were no longer in the earliest and most active disease state by the time they were included in the current study. The best explanation for this observation seems to be that physicians at the 4 centers were reluctant to expose patients with UA to a 1-year course of MTX treatment, given the high spontaneous remission rate and the risk of unnecessary toxicity (3;20). Also, the use of the ACR 1958 criteria for RA as inclusion criteria could have resulted in a selection of UA patients. Despite the fact that UA

patients already had longer disease duration, a 1-year course of MTX treatment was still able to provide beneficial effects on disease and joint damage progression.

The existence of a therapeutic window of opportunity in UA patients, defined as a period of time in which the disease process can be reversed, might not be demonstrated in this study. However, it is possible that with a different design and a different medication scheme, such a therapeutic window can be addressed. First, the incidence of RA increased during tapering of the study medication in the MTX group. This raises the question of what would have happened if MTX had not been tapered. Second, MTX as monotherapy could not have been sufficient, since in the MTX group, half of the RA patients still developed RA while taking study medication, and 6 patients still showed progression of joint damage. Trials in RA patients have shown that treatment with combination therapy and/or biologic agents is more effective in preventing radiographic joint damage (18;19;21). Finally, dosages of study medication were altered according to the DAS because rheumatologists are generally satisfied and do not intensify therapy when the DAS is <2.4 in RA patients (22;23). It is possible that treatment in UA patients should aim at remission or a lower cutoff value of the DAS.

In this study, 53% of the patients in the placebo group developed RA and 24% achieved spontaneous remission, demonstrating that MTX treatment is overtreatment in a considerable proportion of UA patients. Because it is undesirable to start a potentially harmful drug in UA patients who will remit spontaneously, there is a need to identify those UA patients who will most likely develop RA and who will benefit the most from DMARD treatment. In previous studies and in a recently published prediction model that calculates the UA patients' risk of developing RA based on clinical variables, the presence of anti-CCP emerged as one of the strongest predictors of RA (2;4;24). Moreover, applying the model to our study, theoretically initiating treatment in patients with a prediction score ≥8 and withholding treatment in patients with a prediction score ≤6, only 6% of the patients would have been inaccurately withheld from treatment, and no patients would have been inaccurately treated.

In the current study, subgroup analysis revealed that the beneficial outcomes were most pronounced in patients with anti-CCP. In striking contrast, in the anti-CCP-negative subgroup, the effect of MTX on the development of RA, the radiographic progression, and even on the signs and symptoms, was not demonstrable. The same observations were made for patients who were or were not RF-positive, although this could reflect the overlap with anti-CCP. Although the current groups are small, this post hoc analysis suggests that only anti-CCP-positive UA patients, who have the highest risk of developing RA, benefit from early MTX treatment. It also supports the growing evidence that anti-CCP-positive and anti-CCP-negative UA are different disease entities that should be approached differently.

We conclude that treatment with MTX benefits patients with UA by reducing signs and symptoms, by postponing the progression to RA as defined by the ACR 1987 criteria, and by retarding radiographic joint damage. Furthermore, with the guidance of a prediction model and the antibody status, it seems feasible to identify a subset of UA patients who are most in need and who will benefit the most from initiation of MTX therapy, thereby avoiding unnecessary toxic treatment. Although these findings have to be confirmed, and the optimal duration and intensity of treatment still have to be determined, the PROMPT study provides evidence for the efficacy of MTX treatment in UA patients.

Acknowledgements

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CHAPTER 5

Validity of the disease activity score in undifferentiated arthritis

J. Fransen
K. Visser
H. van Dongen
T. Huizinga
P. van Riel
D. van der Heijde

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Summary

Objective

To study whether the disease activity score (DAS) is a valid measure of disease activity in undifferentiated arthritis (UA).

Methods

Data from a double-blind placebo-controlled, randomized trial of MTX and placebo involving 110 patients with UA were used. Data included baseline and 3, 6, 9, and 12 months, and diagnosis at 18 months. Validity of the DAS was analyzed using factor analysis, correlations with disease activity variables, correlations with changes in disability and joint damage, differences in DAS between diagnoses, and detecting the difference between placebo and MTX.

Results

Three disease activity factors were retrieved from the disease activity variables: patient reported outcomes, tender and swollen joints, and acute phase reactants. The DAS had its highest correlations ($r>0.77$) with tender joint counts, followed by swollen joint counts ($r>0.63$) and patient reported outcomes ($r>0.30$), but correlated less with CRP ($r=0.32$). DAS over time was related to HAQ response with an OR (95%CI) of 4.1 (2.1-8.0), but not with change in joint damage. At 18 months, the mean DAS for RA patients was 2.6, for UA it was 2.2, and 1.9 for patients in remission ($p=0.001$). The DAS discriminated better than all single variables between MTX and placebo, with a Guyatt's effect size of 0.89.

Conclusion

The DAS appears to be a reasonably valid measure of disease activity for use in UA clinical trials.

Introduction

In Rheumatoid Arthritis (RA), the disease process develops before the diagnosis can be made according to the current diagnostic criteria for RA (1,2). Indeed, depending on the study population, 6-55% of patients with undifferentiated arthritis (UA) may develop RA within 1 year (3). UA is defined as an inflammatory arthritis in which no definitive diagnosis can (yet) be made. UA may be successfully treated before it develops into erosive arthritis, such as RA (4). Until now, there is no valid measure of disease activity in UA available. The Disease Activity Score (DAS) is a valid measure of RA disease activity (5). The DAS may also be useful and valid in UA clinical trials, but the DAS has not been formally tested in that setting.

The DAS was developed in RA patients, using data from the first 3 years after diagnosis according to the ACR criteria (5). Although the variables included and the weights applied in the DAS were derived from early RA patients, later validation proved that the DAS is also valid in patients with longer disease duration (6). UA covers a spectrum of disease before the ACR classification criteria are fulfilled, and not all patients with UA will eventually develop RA. Moreover, in UA generally less joints are involved, and the pattern of involvement may differ from RA. Therefore, extended joint counts may be more appropriate than reduced joint counts in UA.

The objective was to study whether the DAS is a valid measure of disease activity in early undifferentiated arthritis, using the data from a randomised placebo-controlled trial. The modified DAS (DAS28), which includes reduced joint counts, was also studied. The alternative DAS28 modifications: Clinical Disease Activity Index (CDAI) or Simple Disease Activity Index (SDAI) could not be calculated due to missing components.

Patients and methods

Trial data

Use was made of data from the "PRObable rheumatoid arthritis: Methotrexate versus Placebo Treatment" (PROMPT) trial that is described in more detail elsewhere (4). In summary, this study was a double-blind placebo-controlled, multi-centre, randomized trial involving 110 patients with early undifferentiated arthritis (UA). Treatment started with MTX (15 mg/week) or placebo tablets, and every 3 months the dose was increased if the DAS was >2.4. After 12 months, the study medication was tapered and discontinued in patients not fulfilling the ACR criteria for RA. When a patient fulfilled the ACR criteria for RA (primary endpoint), the study medication was changed to MTX. The diagnosis at the end of the study and the progression of joint damage were prespecified primary outcomes of this trial, to test whether MTX indeed modifies prognosis in UA (4).

Participants and assessments

Eligible patients attended the rheumatology out-clinic of the participating hospitals and had symptoms of arthritis for not longer than 2 years, were at least 18 years of age, and were diagnosed as having UA according to the American College of Rheumatology (ACR) 1958 criteria for probable RA (7).

At baseline and 3, 6, 9, and 12 months, ESR and CRP, the 68 tender joint count, Ritchie Articular Index, 66 swollen joint count, disability measurement (HAQ), and visual analogue scales (0-100) for assessing pain, global disease activity, severity of morning stiffness, fatigue, and general health were obtained. Every 6 months, radiographs of the hands and feet were obtained, and scored according to the Sharp/van der Heijde scoring method. At 18 months, the diagnosis was recorded by the patients' rheumatologist as RA, UA or remission. Remission was defined as no clinical symptoms of arthritis and no DMARD use the preceding year.

The disease activity score was calculated according to the original formula:

$$DAS=0.54x\sqrt{RAI} + 0.065xSJC+0.33xlnESR+0.0072xGH,$$

where RAI is the Ritchie Articular Index, SJC is a 44 swollen joint count, ESR is the Erythrocyte Sedimentation Rate, and GH is a patient assessed visual analogue scale of General Health (0-100 mm.) (5). The RAI contains 53 joints and joint units that are rated for tenderness on a scale from 0 (no tenderness), 1 (pain on pressure), 2 (pain and winced), 3 (winced and withdrew). (8) For the 44 SJC, joints included in the RAI are scored for swelling on a 0-1 scale, excluding TMJ joints, neck, hips, subtarsal and tarsal joints. Levels of DAS<2.4 are equated with "low" disease activity, and levels of DAS>3.7 are equated with "high" disease activity (9). Similarly, the DAS28 was calculated using the formula based on the 28-swollen and 28-tender joint count. (6).

Statistical analysis

Cumulative frequency plots of the 68 tender joint count (TJC68), the 53 joint count of the RAI without grading (TJC53), the 28 tender joint count (TJC28), and the 66, 44 and 28 swollen joint counts (SJC66, SJC44, SJC28) were produced to analyze the degree of misclassification when using reduced joint counts. Differences between SJC66 and SJC44, SJC44 and SJC28 at baseline and at 12 months were tested using the signed rank test; similarly for TJC68 and TJC53, TJC53 and TJC28.

Analysis of the validity of the DAS was performed in 5 steps. For these analyses, data were used from baseline and 3, 6, 9, and 12 months, and the final diagnosis obtained at 18 months. For comparison, the DAS28 was analyzed in the same way.

1) Factor analysis. The DAS had been devised to draw information from three underlying factors of disease activity in RA: physical examination, laboratory values, and patient reported outcomes (5). To reveal the factors underlying assessment of disease activity

in UA, factor analysis with varimax rotation was used with a minimum eigen value >1 to obtain factors using baseline data.

2) Concurrent validity. To inform how strong changes in the DAS within a patient are correlated with changes in other variables supposed to assess disease activity, within-subject correlations were calculated using linear regression with correction for repeated observations, using the 5 visits of the first 12 months (10).

3) Construct validity. It was studied whether the DAS was related to changes in disability and to progression of joint damage over the first 12 months. Ordinal logistic regression was used to relate the time-averaged DAS (0-12 months) to the HAQ response at 12 months. The minimal clinical important difference of 0.22 in HAQ score was used to classify patients as 'worsened', 'unchanged', or 'improved' (11). The proportional odds assumption was checked by dichotomising the HAQ response into 'worsened' versus 'unchanged or improved', and 'worsened or unchanged' versus 'improved' and applying two separate logistic regression models. It was checked graphically whether the logits of the dichotomised HAQ responses were linearly related to the time-averaged DAS (12). The Hosmer-Lemeshov test was used to indicate goodness of fit of both logistic regression models ($p>0.05$), and a Chi-Square score test was used to test the proportional odds assumption in the ordinal logistic regression model ($p>0.05$) (12). Time-averaged DAS, time-averaged CRP and the change in modified Sharp score over 0-12 months were analysed using logistic regression with progression > 3 points (yes/no) in modified Sharp score as dependent variable and also using linear regression with change in modified Sharp score as dependent variable.

4) Criterion validity. It was studied whether the mean DAS over the 5 visits in the first 12 months was different for patients who had erosive arthritis, undifferentiated arthritis, or were in remission at visit 6 (18 months), using ANOVA and using ordinal logistic regression. The underlying assumption is that disease activity, and mean DAS, is highest in patients developing erosive arthritis and lowest in patients going into remission. The proportional odds assumptions for the ordinal logistic regression were tested in a similar way as described above for HAQ responses.

5) Responsiveness. Responsiveness, or sensitivity to change, was calculated over the visits at baseline and 12 months, using the Standardised Response Mean (SRM, mean change / standard deviation of change), and Guyatt's responsiveness statistic (mean change $_{\text{active treated group}}$ / standard deviation $_{\text{placebo group}}$). Moreover, the t-value as a measure of discrimination between the placebo and MTX trial arms was calculated.

Results

The values of the disease activity parameters at baseline of the study are shown in ***Table 1***. The majority of the patients were female, the average age was about 50, 25% (27/110) were positive for anti-CCP antibodies, and 39% (39/110) were rheumatoid Factor positive. According to the distributions of the disease activity parameters in table 1, the majority of patients were in moderate-to-low states of disease activity. There were 36% (20/55) and 45% (25/55) patients with a DAS<2.4 at baseline. There were no statistically significant differences between the trial arms at baseline in any of the parameters. The DAS and the DAS28 had a Gaussian distribution (not shown).

Table 1. Disease activity parameters at baseline.

	MTX (n=55)		Placebo (n=55)	
Gender (female), n (%)	35	(64%)	38	(69%)
Age (year), mean (SD)	53	(14)	50	(14)
Symptom duration at first visit (days), median (IQR)	312	(195-507)	263	(169-432)
RF positive, n (%)	20	(36%)	19	(35%)
Anti-CCP positive, n (%)	12	(22%)	15	(27%)
ESR (mm/h), median (IQR)	12	(5-24)	11	(5-25)
CRP (mg/L), median (IQR)	5	(3-11)	5	(3-9)
SJC28, median (IQR)	2	(1-5)	2	(1-3)
SJC44, median (IQR)	3	(2-5)	2	(2-4)
SJC66, median (IQR)	3	(2-5)	3	(2-5)
TJC28, median (IQR)	6	(2-9)	4	(2-7)
TJC53, median (IQR)	11	(4-16)	7	(4-14)
RAI (0-78), median (IQR)	7	(3-10)	5	(2-8)
TJC68, median (IQR)	11	(4-17)	7	(4-14)
Pain (VAS), median (IQR)	44	(18-61)	48	(28-54)
Disease activity (VAS) , median (IQR)	49	(15-60)	52	(29-64)
Morning stiffness severity (VAS), median (IQR)	45	(20-61)	47	(23-62)
Fatigue (VAS), median (IQR)	55	(22-76)	46	(9-72)
General health (VAS), median (IQR)	40	(19-52)	36	(16-55)
HAQ (0-3), median (IQR)	0.75	(0.38-1.13)	0.75	(0.25-1.13)
DAS, mean (SD)	2.72	(0.78)	2.52	(0.76)
DAS28, mean (SD)	3.94	(1.04)	3.72	(1.06)

Values are count (percentage) or median (interquartile range) or mean (SD). RF=rheumatoid factor, Anti-CCP=anti-cyclic citrullinated peptide antibody, ESR=erythrocyte sedimentation rate, CRP=C-reactive protein, SJC=swollen joint count, RAI=Ritchie articular index, TJC=tender joint count, VAS=visual analogue scale, HAQ=disability index of the Health Assessment Questionnaire, DAS=disease activity score.

Cumulative frequency plots

In ***figure 1***, a cumulative frequency plot of the 28, 44, and 66 swollen joint counts is shown at baseline (A). Every individual patient is represented in the curves as a single symbol, patients being ranked by the number of joints involved. The different kind of symbols denote the different joint counts, the patients do not necessarily have the same rank in the three curves. All differences between reduced and extended joint counts were statistically significant ($p \leq 0.002$), for the tender joint counts (B) as well as for the swollen joint counts (A) at baseline, and at 12 months (not shown).

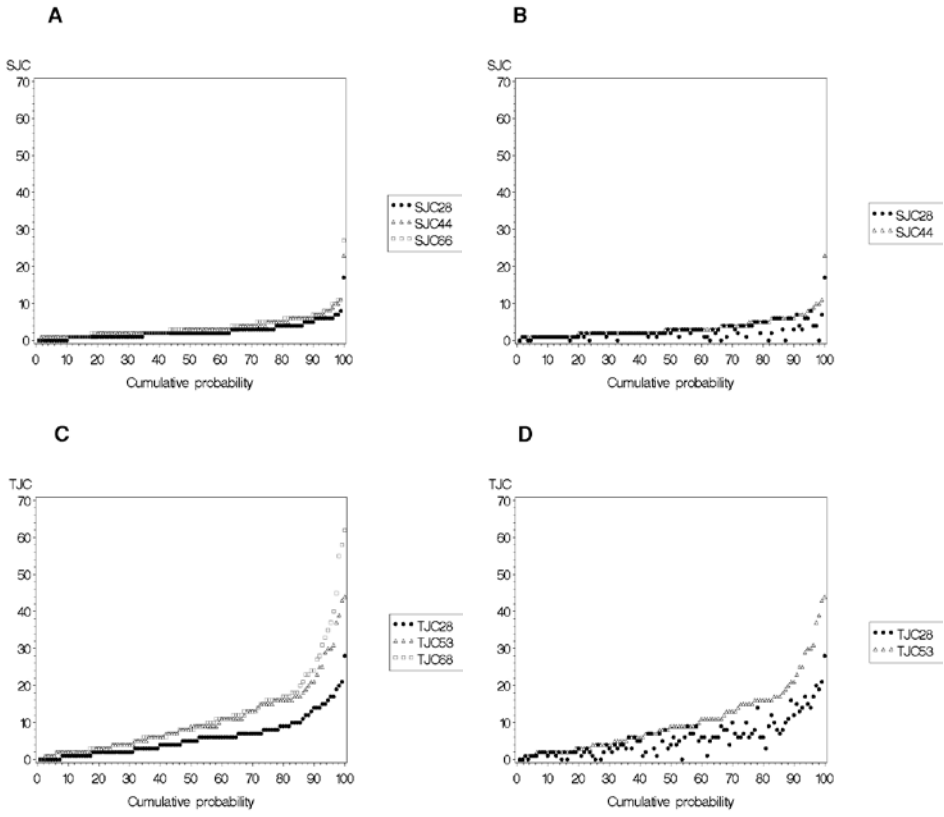

Figure 1. Cumulative frequency plots of joint counts at baseline: average differences (A and B) and individual differences (C and D).
SJC=Swollen Joint Count; TJC=Tender Joint Count. Every individual patient is represented in the plots as a single symbol, patients being ranked by the number of joints involved. In panel A and B, the patients do not necessarily have the same rank in the three curves. The position of the median scores is where the cumulative frequency is 50%. In panel C and D, again every individual patient is represented in the plots as a single symbol, patients being ranked by their scores on the extended joint count. Only the median of the extended joint count is at a frequency of 50%.
The different joint counts are denoted by the different kind of symbols (see legend).

It can be seen in ***figure 1 A*** that the 66 SJC (squares) detects most swollen joints, with the 44 SJC (triangles) being close to it. The 28 SJC (filled dots) scores are somewhat lower than the other scores and the 28 SJC thus slightly underestimates the number of swollen joints. The number of patients with no swollen joints according to the 28 SJC and one or more swollen joints according to the extended joint counts does not exceed 10% at baseline, and at 12 months (not shown). ***Figure 1 B*** similarly shows the cumulative frequency plots of the 28, 53, and 68 tender joint counts at baseline. It can be seen that the number of tender joints involved is larger than the number of swollen joints. In figure 1 B, the scores of the tender joint counts follow the same trend, but the 28 TJC is the lowest. Misclassification (no tender joints according to the 28 TJC but at least 1 tender joint according to the extended joint counts occurred in 6% at baseline (1 B) and 11% at 12 months.

In ***figure 1 C and D***, cumulative frequency plots are shown of the 28 and 44 SJC (C) and the 28 and 53 TJC (D). Now the patients are ordered by their scores on the extended joint count only. It can be seen in figure C that patients with no or few swollen joints according to the 28 SJC may have more than 1 swollen joint according to the 44 SJC. The same is seen for the 28 and 53 TJC in figure D.

Table 2. Exploratory factor analysis at baseline.

	Factor		
	1	**2**	**3**
ESR (mm/h)	-0.05	0.02	0.88
CRP (mg/L)	-0.02	-0.09	0.88
SJC44	-0.15	0.60	-0.03
TJC53	0.18	0.93	-0.06
RAI (0-78)	0.28	0.85	-0.03
Pain (VAS)	0.86	0.06	0.10
Disease activity (VAS)	0.82	-0.08	0.08
Morning stiffness (VAS)	0.65	0.29	-0.14
Fatigue (VAS)	0.72	0.29	-0.12
General health (VAS)	0.85	-0.01	-0.13
HAQ (0-3)	0.53	0.57	0.06
Variance explained	46%	32%	22%

Three factors were derived using exploratory factor analysis with varimax rotation and a mineigen value >1 to obtain factors using baseline data. The factors may be named 1) 'patient reported outcomes', 2) 'joint counts', and 3) 'acute phase reactants'. For abbreviations see Table 1.

Factor analysis

By factor analysis, three factors were retrieved from the disease activity parameters at baseline ***(Table 2)***. The first factor consisted of all the items on patient reported outcomes, the second factor consisted of the examinations of tender and swollen joints, the third factor included the acute phase reactants ESR and CRP. The HAQ loaded on both the first and second factors. The ordering of factors in factor analysis and the amount of variance explained also reflect the number of items included.

Concurrent validity

In ***table 3***, the within-subject correlations of the DAS with other disease activity variables are shown, using the visits of the first 12 months. The DAS had its highest correlations with the RAI and the tender joint counts, it correlated well with the swollen joint counts and most of the patient assessments and correlated less with CRP. Correlations of the DAS28 generally were similar.

Table 3. Within-subject correlations with DAS and DAS28 over visits 1-5.

	DAS		DAS28	
	r	**(95%CI)**	**r**	**(95%CI)**
ESR (mm/h)	0.47	(0.31-0.61)	0.50	(0.34-0.63)
CRP (mg/L)	0.32	(0.14-0.48)	0.36	(0.19-0.52)
SJC28	0.62	(0.49-0.72)	0.63	(0.50-0.73)
SJC44	0.63	(0.50-0.73)	0.52	(0.37-0.65)
SJC66	0.62	(0.49-0.72)	0.50	(0.35-0.63)
TJC28	0.62	(0.49-0.72)	0.69	(0.58-0.78)
TJC53	0.71	(0.60-0.79)	0.63	(0.51-0.73)
RAI (0-78)	0.77	(0.68-0.84)	0.60	(0.47-0.71)
TJC68	0.64	(0.51-0.74)	0.57	(0.43-0.69)
Pain (VAS)	0.43	(0.26-0.57)	0.49	(0.33-0.62)
Disease activity (VAS)	0.43	(0.27-0.57)	0.48	(0.32-0.61)
Morning stiffness (VAS)	0.39	(0.22-0.54)	0.42	(0.25-0.56)
Fatigue (VAS)	0.30	(0.12-0.46)	0.35	(0.17-0.50)
General health (VAS)	0.46	(0.30-0.60)	0.54	(0.39-0.66)
HAQ (0-3)	0.35	(0.17-0.50)	0.35	(0.21-0.53)

The within-subject correlations inform about in how far a change in DAS (or DAS28) is paralelled by a change in the variable in the left-hand column. For abbreviations see Table 1.

Construct validity

It was studied whether the time averaged DAS over 12 months was associated with a change in HAQ over the same period of time, irrespective of treatment allocation ***(Figure 2)***. HAQ scores were available of 102 patients. Over 12 months, there were 16

patients with a worsening in HAQ over 0.22 points, 40 patients with an improvement over 0.22, and 46 patients with smaller changes in either direction who were regarded as 'unchanged' (Figure 2). In ordinal logistic regression, the time averaged DAS was significantly associated with a minimal clinical important change (>0.22) in HAQ, with an OR (95%CI) of 4.1 (2.1-8.0), corrected for baseline HAQ, anti-CCP, age and gender as confounders. The assumptions for ordinal logistic regression, as outlined in the methods, were met. Further, it was studied whether time averaged disease activity and joint damage progression were correlated. However, there was no relation between time averaged CRP or time averaged DAS and change in Sharp score, irrespective of RF positivity, anti-CCP positivity, treatment, or final disease outcome (results not shown).

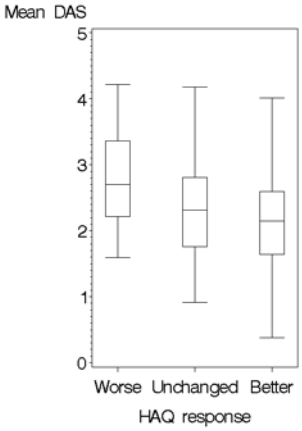

Figure 2. Response in HAQ and DAS scores averaged over time.
Box plots of the patient-averaged DAS over month 0-12, by HAQ response at month 12. HAQ response was defined as worsening (change in HAQ score > -0.22), no change (change in HAQ score between -0.22 and +0.22), and improvement (change in HAQ score > +0.22), according to (11).

Criterion validity

The level of disease activity is supposedly different for patients who develop RA, have undifferentiated arthritis, or reach remission. The mean (SD) DAS for patients with RA (n=49) was 2.6 (0.90), for patients with undifferentiated arthritis (n=21) it was 2.2 (0.72), for patients reaching remission (n=29) the DAS was 1.9 (0.64); p=0.001 for the differences between diagnostic groups. The OR (95%CI) for the differences between diagnostic groups in ordinal logistic regression was 2.5 (1.5-4.2) per time-averaged DAS point. A similar difference was observed for the DAS28. The assumptions for ordinal logistic regression were met for both analyses.

The mean (SD) DAS28 for patients with RA was 3.7 (1.09), for patients with undifferentiated arthritis it was 3.2 (1.91), for patients reaching remission the average DAS28 over time was 2.8 (0.79); p=0.0007 for the differences between diagnostic groups. At

18 months, 13 patients fulfilled the ARA criteria of remission. At that time, there were 32 patients with a DAS<1.6, and 13/32 (40%) fulfilled the ARA criteria for remission. The number of patients with a DAS28<2.6 was 37, with 13/37 (35%) fulfilling the ARA criteria.

Responsiveness

The changes in disease activity parameters from baseline to 12 months are shown in Table 4. Disease activity improved in both treatment arms, but generally changes were larger in the MTX group. When adopting p=0.05 as critical value for determining statistical significance, the critical value of t with this sample size is 2.0. In table 4 it can be seen that in the MTX group the DAS had a higher responsiveness (SRM) than the single parameters. The tender joint counts and the DAS and DAS28 showed the best performance in discriminating MTX from placebo according to Guyatt's responsiveness statistic and the t-value, with increasing discrimination with increasing number of joints assessed. The other variables did not exceed the critical t-value value of 2.0.

Table 4. Responsiveness of disease activity parameters.

	MTX (n=55)			Placebo (n=55)				
	Mean	**(SD)**	**SRM**	**Mean**	**(SD)**	**SRM**	**Guyatt's ES**	**t-value**
DAS	-0.8	(0.8)	1.02	-0.4	(0.9)	0.40	0.89	2.40
DAS28	-1.1	(1.1)	1.05	-0.6	(1.3)	0.47	0.85	2.15
ESR (mm/h)	-5.6	(11.8)	0.47	-1.8	(15.2)	0.12	0.37	1.40
CRP (mg/L)	-3.6	(15.0)	0.24	-0.7	(17.0)	0.04	0.21	0.87
SJC28	-1.7	(2.6)	0.62	-1.2	(2.9)	0.42	0.57	0.77
SJC44	-1.9	(3.6)	0.53	-1.0	(4.7)	0.20	0.55	1.17
SJC66	-2.3	(4.5)	0.52	-1.4	(4.7)	0.29	0.55	1.08
TJC28	-2.7	(3.9)	0.68	-0.5	(5.2)	0.10	0.52	2.44
TJC53	-3.8	(6.4)	0.59	-0.31	(6.8)	0.04	0.57	2.71
RAI (0-78)	-3.3	(5.0)	0.65	-0.7	(4.4)	0.16	0.74	2.77
TJC68	-4.8	(7.8)	0.62	0.17	(9.0)	0.02	0.54	3.04
Pain (VAS)	-11	(25)	0.45	-15	(28)	0.52	0.41	0.59
Disease activity (VAS)	-10	(24)	0.41	-17	(28)	0.59	0.34	1.39
Morning stiffness (VAS)	-13	(25)	0.53	-15	(28)	0.54	0.46	0.49
Fatigue (VAS)	-7	(25)	0.27	-7	(30)	0.24	0.22	0.10
General health (VAS)	-9	(21)	0.44	-6	(31)	0.20	0.29	0.54
HAQ (0-3)	-0.19	(0.45)	0.42	-0.13	(0.47)	0.29	0.40	0.59

Shown are changes over 0-12 months in mean (SD). SRM=standardised response mean. For other abbreviations see Table 1.

Discussion

In this post-hoc analysis of the data from the PROMPT study of MTX versus placebo in patients with early UA, it was studied whether the DAS is a valid measure of disease activity in early UA. According to the results of this study, the DAS indeed appears to be a reasonably valid measure of disease activity for use in UA clinical trials. However, the validity of the DAS to measure arthritic disease activity in UA should also be tested in other UA samples in different settings.

In absence of an unambiguous "gold standard" measure, validation took five steps: factor analysis, concurrent validity, construct validity, criterion validity and responsiveness. The primary interest of this study in UA was in the DAS, because it includes extended joint counts. The DAS28 with its reduced (28) joint counts was included to be able to see whether or not there were large differences between the two measures in their performance.

According to the comparison of reduced with extended joint counts in cumulative frequency plots, it appeared that up to 10% of patients were misclassified as having no swollen or tender joints by the 28 joint count. In contrast, these patients had 1 to 10 joints involved according to the extended joint counts. The largest difference between 28 and extended joint counts is that the former does not include the feet joints. Feet joints may typically be involved in early RA (13). As the DAS includes extended joint counts, the DAS may be preferred over the DAS28 in clinical trials in early RA or UA. It could be shown in the frequency plots that the reduced joints are underestimating the involvement of the number of joints. However, the lesser performance of the reduced joint counts was not substantiated in the correlation of the DAS28 with other variables and its responsiveness.

By the factor analysis, similar factors of disease activity were found in this study as previously in RA (5). The three factors were patient reported outcomes, tender joints and swollen joints, and acute phase reactants. Indeed, as can be seen in the DAS formula, the DAS taps information from each of these constructs. Results of factor analysis depend on the variables that are put in. The fact that a large proportion of the disease activity parameters was patient-assessed, that naturally are closely correlated, will have contributed to retrieval of patient reported outcomes as the first factor.

In this study, the DAS correlated moderate-to-good with all other disease activity parameters, including patient assessments, except for the acute phase reactants. The contribution of the GH item to the DAS is relatively low (maximally 0.72 if GH is scored 100), which may be criticized as neglecting the patient perspective. However, despite the weighting, the DAS is reasonably well associated with the other frequently used patient assessments. The low within-subject correlations of the DAS with ESR and CRP will be caused by the large proportion of patients who had acute phase reactants in

the normal range so changes in the acute phase reactants were not highly related to changes in DAS or DAS28. On the other hand it is known that the DAS28 is sensitive for changes in the low (even normal) range of ESR (14). The DAS over time was indeed related to a change in HAQ, as expected in advance. However, DAS over time and CRP over time were both not associated with the progression of joint damage in this study. This could not be explained, not even approximately, by differences in RF positivity, anti-CCP positivity, treatment, or final disease outcome. However, the small number of patients experiencing joint damage progression made it difficult to analyse these relations.

It was supposed in advance that the course of disease activity would be different for patients who turned out to have RA, who still had UA, or who were in remission at the end of the study. Indeed the mean DAS levels were clearly different for these three diagnostic groups. The DAS and the DAS28 both did not perform well in predicting the ARA criteria for clinical remission: all patients with ARA clinical remission had a DAS<1.6 and a DAS28<2.6, but only 40% of patients with a DAS<1.6 and 35% of patients with a DAS28<2.6 fulfilled the ARA remission criteria.

The original PROMPT trial was powered for the primary outcome, which was the diagnosis reached at 18 months (4). When analyzing the responsiveness of the several disease activity parameters included as secondary outcomes, the DAS discriminated well between MTX and placebo. Neither of the other parameters, except the tender joint counts, reached statistical significance. Like in RA clinical trials, the pooling of information in the DAS therefore may be advantageous for trials in UA.

An advantage of the DAS for assessment of disease activity is that it includes extended joint counts. Whilst the 28 joint counts were introduced to facilitate a quick performance of joint counts, their use may lead to an underestimation of disease activity if only few joints are involved that are not among the 28 counted, such as the feet. This may play a role in UA as well as in early RA. In UA, disease activity is generally low, and by using extended joint counts including the feet an underestimation of the number of joints involved may be prevented. For that reason, for clinical trials of early interventions in UA or RA that aim at clinical remission the DAS may be preferred over the DAS28 (13). On the other hand, in this validation study in UA, the DAS28 performed closely to the DAS regarding all parameters of validity and responsiveness. It was the assessment of clinical remission that was impeded by the use of reduced joint counts.

In summary, it can be concluded that the DAS, which is a valid measure of RA disease activity, appears to be a reasonably valid measure of disease activity for use in UA clinical trials. Similar factors of disease activity were found in this study as previously in RA; the DAS correlated moderate-to-good with all other disease activity parameters including patient-assessments; the DAS over time was related to changes in HAQ; the DAS was different for patients with RA, UA and patients in remission; and the DAS discriminated well between MTX and placebo. It may be regarded that the PROMPT trial we used was

relatively small (N=110) for validation purposes and therefore, studying the validity of the DAS in UA in other samples is most welcome. The DAS may be used as primary or secondary outcome measure for clinical trials in early UA. Instead of the DAS, the DAS28 may also be used, but the use of reduced joint counts underestimates the involvement of inflamed joints.

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CHAPTER 6

A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis. How to guide individual treatment decisions

A.H.M. van der Helm-van Mil
S.le Cessie
H. van Dongen
F.C. Breedveld
R.E.M. Toes
T.W.J. Huizinga

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Abstract

Objective

In patients with undifferentiated arthritis (UA), methotrexate is effective for inhibiting symptoms, structural damage, and progression to rheumatoid arthritis (RA). However, 40-50% of patients with UA experience spontaneous remission. Thus, adequate decision-making regarding treatment of patients with early UA requires identification of those patients in whom RA will develop.

Methods

A prediction rule was developed using data from the Leiden Early Arthritis Clinic, an inception cohort of patients with recent-onset arthritis (n = 1,700). The patients who presented with UA were selected (n = 570), and progression to RA or another diagnosis in this group was monitored for 1 year of followup. The clinical characteristics with independent predictive value for the development of RA were selected using logistic regression analysis. The diagnostic performance of the prediction rule was evaluated using the area under the curve (AUC). Cross-validation controlled for overfitting of the data (internal validation). An independent cohort of patients with UA was used for external validation.

Results

The prediction rule consisted of 9 clinical variables: sex, age, localization of symptoms, morning stiffness, the tender joint count, the swollen joint count, the C-reactive protein level, rheumatoid factor positivity, and the presence of anti-cyclic citrullinated peptide antibodies. Each prediction score varied from 0 to 14 and corresponded to the percent chance of RA developing. For several cutoff values, the positive and negative predictive values were determined. The AUC values for the prediction rule, the prediction model after cross-validation, and the external validation cohort were 0.89, 0.87, and 0.97, respectively.

Conclusion

In patients who present with UA, the risk of developing RA can be predicted, thereby allowing individualized decisions regarding the initiation of treatment with disease-modifying antirheumatic drugs in such patients.

Introduction

Making individualized decisions regarding treatment is one of the most important challenges in medicine. To this end, several studies have associated clinical variables or gene expression profiles with disease outcome, thereby providing help for clinicians making treatment decisions in several diseases, e.g., breast cancer, Hodgkin's disease, and lymphoma (1-4). For the past decennium, treatment of rheumatoid arthritis (RA) has been characterized by early, aggressive treatment with disease-modifying antirheumatic drugs (DMARDs), because this treatment strategy prevents joint damage and functional disability (5-7).

In rheumatology practices, the majority of patients who present with recent-onset arthritis have undifferentiated arthritis (UA), which is a form of arthritis that does not fulfill the classification criteria for a more definitive diagnosis. Based on results from several inception cohort studies, it is known that ~40-50% of patients with UA experience spontaneous remission, whereas RA develops in one-third of patients with UA (8-10). Recent evidence indicates that treatment with methotrexate in patients with early UA hampers progression to RA and progression of joint damage (11), underscoring the need for guidance when starting treatment with a clinically beneficial but potentially harmful drug in UA. Ideally, only the patients with UA in whom RA develops would be treated with DMARDs, excluding those in whom UA remits spontaneously.

At present, although several risk factors for the development of RA have been identified (8;12), a model that predicts the disease course specifically in patients with recent-onset UA is lacking. In the present study, we aimed to develop a model that predicts progression from UA to RA, using clinical variables that are easily assessed in clinical practice. The derived prediction rule was internally validated, controlling for overfitting of the data, and was subsequently externally validated in an independent cohort of patients with UA

Patients and methods

Patients

The prediction rule was derived using the Leiden Early Arthritis Clinic (EAC) cohort. This inception cohort comprises more than 1,900 patients with recent-onset arthritis, of whom ~1,700 have completed at least 1 year of followup. The EAC cohort began in 1993 at the Department of Rheumatology of the Leiden University Medical Center, the only referral center for rheumatology in a health care region of ~400,000 inhabitants in The Netherlands (13). General practitioners were encouraged to refer patients directly

when arthritis was suspected; patients were included if a physical examination revealed arthritis.

At the first visit, the rheumatologist completed a questionnaire regarding the presenting symptoms, as reported by the patient: type, localization and distribution of initial joint symptoms, symptom duration, and course of the initial symptoms. The patient's smoking history and family history were assessed. Patients rated morning stiffness on a visual analog scale (VAS; range 0-100 mm). For the present study, the severity of morning stiffness was used instead of the duration of morning stiffness, because the former variable has been proven to be a better discriminator (14;15). The Health Assessment Questionnaire (16) was used to provide an index of disability. A 44-joint count for tender and swollen joints was performed, scoring each joint on a 0-1-point scale (17). Compression pain in the metacarpophalangeal and metatarsophalangeal joints was recorded.

Baseline blood samples were obtained for determination of the erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP) level, the presence of IgM rheumatoid factor (RF), as determined by enzyme-linked immunosorbent assay (ELISA), and the presence of antibodies to cyclic citrullinated peptide 2 (anti-CCP), as determined by ELISA (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff level for anti-CCP positivity was 25 arbitrary units. Radiographs of the hands and feet were obtained and scored according to the Sharp/van der Heijde method (18). Patients provided informed consent, and the local ethics committee approved the protocol.

Assessment of disease status after 1 year

Two weeks after being included in the study, when results of laboratory investigations and radiography were known, 570 patients were determined to have a form of arthritis that could not be classified according to American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria (19) and were documented as having UA. After 1 year of followup, the disease status of all patients with UA was examined to determine whether RA or another specific type of arthritis had developed, based on fulfillment of the ACR criteria. Inherent in the design of an inception cohort, the duration of followup differed within the study population, and at the moment of analysis (July 2005), the majority of patients with UA (94%) had been followed up for more than 1 year (mean ± SD followup 8 ± 3 years).

External validation cohort

Patients included in the placebo arm of the Probaat (PROMPT) trial, a double-blind, placebo-controlled, randomized trial in which patients with recent-onset UA were treated with either methotrexate or placebo, were used for validation (n = 55) (11). Exclusion of the patients with UA who were also included in the EAC cohort resulted in 36 independent patients with UA. Two of these patients were lost to followup. For each

patient, the progression score at baseline was calculated, and the development of RA after 1 year of followup was assessed (11).

Statistical analysis

Patients with UA in whom RA developed were compared with those in whom RA did not develop, using the chi-square test for nominal variables and Student's t-test for continuous variables. Symptom duration was categorized. Subsequently, all clinical variables were entered as possible explanatory variables in a logistic regression analysis, with disease outcome (RA or non-RA) at 1 year of followup as the dependent variable. Using a backward selection procedure, the most significant independent variables were identified, using a *P* value greater than 0.10 as the removal criterion.

In the logistic regression model, the predicted probability of RA was related to the covariates via the following prognostic index: $B1 \times x1 + B2 \times x2 + B3 \times x3 \ldots Bk \times xk$. The regression coefficient (B) of the covariate indicates an estimate of the relative magnitude of the prognostic power of a specific variable. Using the prognostic index, we calculated the predicted probability of RA developing for every patient. For continuous variables (age, VAS score, tender and swollen joint counts, CRP level), the effect was studied both as a continuous variable and as a categorized variable. Categories were created using clinically applicable cutoff levels and percentiles. Categories were pooled if corresponding regression coefficients were similar. Data were missing for some patients, as follows: morning stiffness score on a 100-mm VAS (n = 160 patients), anti-CCP antibody level (n = 64 patients), disease duration (n = 22 patients), tender joint count (n = 5 patients), swollen joint count (n = 4 patients), CRP level (n = 1 patient), and presence of RF (n = 1 patient). To prevent exclusion of these patients from the logistic regression analysis, the median values for these variables were imputed.

To obtain a simplified prediction rule, the regression coefficients of the predictive variables were rounded to the nearest number ending in .5 or .0, resulting in a weighted score; subsequently, the values for the independent predictive variables were summed. The calculated prediction scores were compared with the observed percentage of patients who experienced progression to RA. The positive and negative predictive values were determined for several cutoff values of the prediction scores. To evaluate the diagnostic performance of the rule, a receiver operating characteristic (ROC) curve was constructed. The area under the ROC curve (AUC) values provided a measure of the overall discriminative ability of a model.

For internal validation, cross-validation was performed to control for overfitting (20). Cross-validation mimics the prediction situation and for each observation yields a prediction score based on the other (n - 1) observations (20). To validate the model, a ROC curve was made using the cross-validated predictions as well as the external validation cohort. SPSS version 10.0 software (SPSS, Chicago, IL) was used.

Table 1. Baseline characteristics of patients with UA, according to progression to RA*

Characteristic	No progression to RA (n = 393)	Progression to RA (n = 177)	*P*
Age, mean ± SD	48.6 ± 17.0	56.3 ± 15.3	<0.001
Female sex	208 (53)	121 (68)	0.001
Positive family history of RA	81 (21)	54 (31)	0.01
Course of starting symptoms			
acute for <24 hours	116 (30)	36 (20)	
subacute for > 24 hours	123 (31)	51 (29)	
gradual	141 (36)	86 (49)	
intermittent	13 (3)	4 (2)	0.02
Symptom duration at inclusion			
< 6 weeks	103 (27)	18 (11)	
6 weeks to 3 months	80 (21)	43 (25)	
3 to 6 months	89 (23)	47 (28)	
> 6 months	107 (28)	61 (36)	<0.001
Localization of affected joints			
small joints	171 (44)	95 (54)	
large joints	165 (42)	32 (18)	
small and large joints	57 (15)	50 (28)	<0.001
Localization of affected joints			
symmetric	147 (37)	118 (67)	<0.001
Localization of affected joints			
upper extremities	177 (45)	71 (40)	
lower extremities	139 (35)	22 (12)	
upper and lower extremities	77 (20)	84 (47)	<0.001
Morning stiffness, mean ± SD score on a 100-mm VAS	35.5 ± 30.0	53.3 ± 30.1	<0.001
Compression pain in MCP joints	159 (40)	116 (66)	<0.001
Compression pain in MTP joints	134 (34)	103 (58)	<0.001
Number of tender joints, median (IQR)	5 (2-11)	11 (7-22)	<0.001
Number of swollen joints, median (IQR)	2 (1-4)	4 (2-7)	<0.001
C-reactive protein level, median (IQR) mg/liter	8 (3-21)	14 (7-43)	<0.001
ESR, median (IQR) mm/hour	17 (8-38)	32 (19-53)	<0.001
Rheumatoid factor positivity	56 (14)	84 (47)	<0.001
Anti-CCP positivity	38 (11)	83 (51)	<0.001
HAQ score, mean ± SD	0.7 ± 0.6	1.0 ± 0.7	<0.001
Smoking	187 (48)	84 (47)	1.0
Erosive disease	29 (7)	29 (16)	0.001

* Except where indicated otherwise, values are the number (%). UA = undifferentiated arthritis; RA = rheumatoid arthritis; VAS = visual analog scale; MCP = metacarpophalangeal; MTP = metatarsophalangeal; IQR = interquartile range; ESR = erythrocyte sedimentation rate; anti-CCP = anti-cyclic citrullinated peptide; HAQ = Health Assessment Questionnaire.

Results

Disease outcome

During the first year of followup, RA developed in 177 of the 570 patients with UA, other rheumatologic disease developed in 94 patients, and 150 patients achieved clinical remission, defined as discharge from the outpatient clinic because of the absence of arthritis while not receiving DMARD treatment. For further analysis, patients with another rheumatologic diagnosis or UA and those who achieved remission were assembled as the non-RA group (n = 393).

Table 2. Independent predictive variables for development of RA based on results of multivariate regression analysis*

Variable	B	OR	95%CI	*P*	Points†
Sex	0.8	2.1	1.3-3.6	0.003	1
Age	0.02	1.02	1.01-1.04	0.011	0.02/year
Localization small joints hand/feet	0.6	1.8	1.1-3.1	0.024	0.5
Symmetric localization	0.5	1.6	1.0-2.8	0.075	0.5
Localization in upper extremities	0.8	2.1	1.1-4.4	0.04	1
Localization in both upper and lower extremities	1.3	3.5	1.7-7.5	0.001	1.5
Morning stiffness score on 100-mm VAS					
0-25	-	-	-	-	-
26-50	0.9	2.4	1.2-4.5	0.009	1
51-90	1.0	2.7	1.3-5.6	0.006	1
>90	2.2	9.3	3.0-28.7	<0.001	2
Number of tender joints					
0-3	-	-	-	-	-
4-10	0.6	1.8	0.9-3.3	0.082	0.5
>10	1.2	3.3	1.5-7.0	0.003	1
Number of swollen joints					
0-3	-	-	-	-	-
4-10	0.4	1.5	0.8-2.7	0.18	0.5
>10	1.0	2.8	1.1-7.6	0.038	1
CRP level, mg/liter					
0-4	-	-	-	-	-
5-50	0.6	1.6	0.9-3.0	0.13	0.5
>50	1.6	5.0	2.0-12.1	0.00	1.5
RF positivity	0.8	2.3	1.2-4.2	0.009	1
Anti-CCP positivity	2.1	8.1	4.2-15.8	<0.001	2

* B values are regression coefficients. RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval; VAS = visual analog scale; CRP = C-reactive protein; RF = rheumatoid factor; anti-CCP = anti-cyclic citrullinated peptide.

† For the simplified prediction rule derived from the regression coefficient.

Univariate analyses

The characteristics of patients with UA in whom RA developed and those in whom RA did not develop are compared in ***Table 1***. In the univariate analyses, all variables except smoking were significantly associated with progression to RA.

Multivariate analyses and derivation of the prediction rule

In the logistic regression analysis, the independent predictive variables for development of RA were age, sex, localization of joint symptoms (small/large joints, symmetric/asymmetric, upper/lower extremities), morning stiffness, tender and swollen joint counts, CRP level, and the presence of RF or anti-CCP antibodies (Table 2).

Because age was more predictive as a continuous variable than as a categorized variable, age was not categorized. The other variables were categorized. For the resulting model, the fraction of explained variation (Nagelkerke's R2) was 0.57; when using a predicted probability of 0.5 as the cutoff value, the outcomes for 83% of patients were predicted correctly. The coefficients for the simplified prediction score are listed in ***Table 2***. ***Figure 1*** presents a form that can be used to easily calculate the prediction score. The

1. What is the age in years?	Multiply with 0.02		_____
2. What is the sex?			
	In case female:	1 point	_____
3. How is the distribution of involved joints?			
	In case small joints hands/feet:	0.5 point	_____
	In case symmetric:	0.5 point	_____
	In case upper extremities	1 point	_____
	In case upper and lower extremities:	1.5 points	_____
4. What is the score for morning stiffness on a 100-mm VAS?			
	In case 26-90 mm:	1 point	_____
	In case >90 mm:	2 points	_____
5. What is the number of tender joints?			
	In case 4-10:	0.5 point	_____
	In case 11 or higher:	1 point	_____
6. What is the number of swollen joints?			
	In case 4-10:	0.5 point	_____
	In case 11 or more:	1 point	_____
7. What is the C-reactive protein level?			
	In case 5-50 mg/liter:	0.5 point	_____
	In case 51 mg/liter or higher:	1.5 points	_____
8. Is the patient rheumatoid factor positive?			
	If yes:	1 point	_____
9. Are the anti-CCP antibodies positive?			
	If yes:	2 points	_____
		Total score	_____

Figure 1. Form used to calculate a patient's prediction score. The range of possible scores is 0-14, with higher scores indicating a greater risk of developing rheumatoid arthritis. VAS = visual analog scale; anti-CCP = anti-cyclic citrullinated peptide.

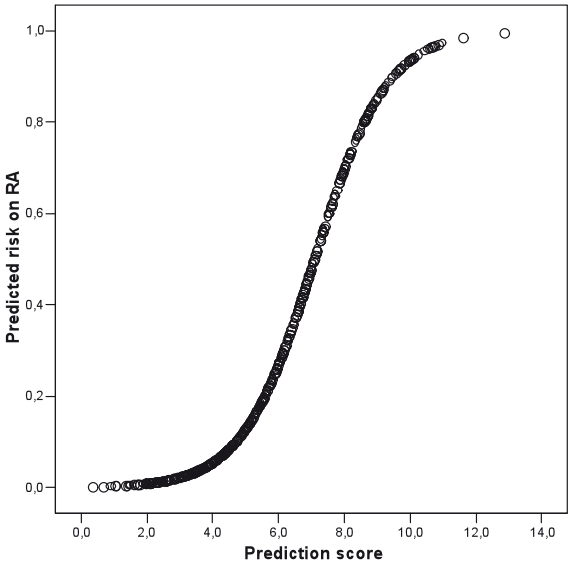

Figure 2. Predicted risk of rheumatoid arthritis (RA) as a function of the prediction score.

range of the prediction scores is 0-14, with a higher score indicating a greater risk of developing RA.

A prediction score was calculated for every patient with UA. ***Figure 2*** shows the predicted risk of RA as a function of the prediction score (obtained from a logistic regression model with score as the independent variable). ***Table 3*** shows the observed percentage of patients who experienced progression to RA in relation to the calculated prediction score. None of the patients with UA who had a prediction score ≤3 progressed to RA during the 1-year followup period, and all of the patients with UA who had a score ≥11 did experience progression to RA. Among the patients with scores of 4-10 who experienced progression to RA, the frequency of such progression increased with rising scores.

Table 4 shows the percentage of patients in whom RA developed, according to several cutoff values of the prediction score. For example, when the scores 5.0 and 9.0 were chosen as cutoff values, 97% of patients with UA who had a score ≤5.0 did not develop RA, and a score ≥9.0 was associated with progression to RA in 84% of patients. When cutoff values of 6.0 and 8.0 were used, 91% of patients with UA who had a score ≤6.0 did not develop RA (negative predictive value 91%, 95% confidence interval [95% CI] 88-94%), and a score ≥8.0 corresponded to progression to RA in 84% of patients (positive predictive value 84%, 95% CI 75-91%). Using these cutoff values, 145 patients with UA (25%) had a score between 6.0 and 8.0, indicating that no adequate prediction could be made for these patients. Twenty-five patients with UA did not fulfill the 1987 ACR criteria for RA after 1 year of followup, but RA developed later in the disease course. These patients had a median prediction score of 5.7 (interquartile range [IQR] 4.8-6.2); this value is between

Table 3. Prediction scores and progression or nonprogression to RA*

Prediction score	No progression to RA (n = 387)	Progression to RA (n = 175)
0	1 (100)	0 (0)
1	8 (100)	0 (0)
2	42 (100)	0 (0)
3	58 (100)	0 (0)
4	78 (93)	6 (7)
5	73 (85)	13 (15)
6	63 (74)	22 (26)
7	37 (49)	38 (51)
8	16 (33)	33 (67)
9	6 (14)	36 (86)
10	5 (23)	17 (77)
11	0 (0)	8 (100)
12	0 (0)	1 (100)
13	0 (0)	1 (100)
14	0 (0)	0 (0)

* Values are the number (%) of patients with a given score. Scores were rounded to the nearest number ending in .5 or .0 (i.e., scores ≤0.5 are in the category 0, scores >0.5 and ≤1.5 are in the category 1, etc.). RA = rheumatoid arthritis.

Table 4. Cutoff values for prediction scores and risk of development of RA*

Cutoff values	No progression to RA	Progression to RA
Score ≤ 4.0	145 (99)	1 (1)
4.0 -10.0	240 (60)	159 (40)
≥10.0	2 (12)	15 (88)
Score ≤ 5.0	223 (97)	8 (3)
5.0-9.0	157 (55)	131 (46)
≥ 9.0	7 (16)	36 (84)
Score ≤ 6.0	296 (91)	28 (9)
6.0-8.0	76 (52)	69 (48)
≥8.0	15 (16)	78 (84)

* Values are the number (%) of patients with a given score. Scores were rounded to the nearest number ending in .5 or .0. RA = rheumatoid arthritis.

the scores for the patients with UA in whom RA developed and those in whom RA did not develop during the first year of followup (median score 7.7 [IQR 6.6-8.8] and median score 4.6 [IQR 3.3-5.9], respectively).

Discriminative ability

The discriminative ability of the logistic regression model and the prediction rule were evaluated with a ROC curve (Figure 3). Both the logistic regression model and the pre-

diction rule had a mean ± SD AUC value of 0.89 ± 0.014. The finding that the AUC values for the logistic regression model and the prediction rule were equal indicates that the derivation of the prediction rule from the logistic regression model had not introduced a loss of discriminative ability.

Internal validation

Cross-validation was used to control for overfitting. This procedure yielded a value for the predicted probability of RA for every patient, based on results of model-fitting on the other patients (20). The AUC value of the cross-validated predictions nearly equaled the mean ± SD AUC value of the prediction score (0.87 ± 0.015) (***Figure 3***), indicating that overfitting was not a major problem.

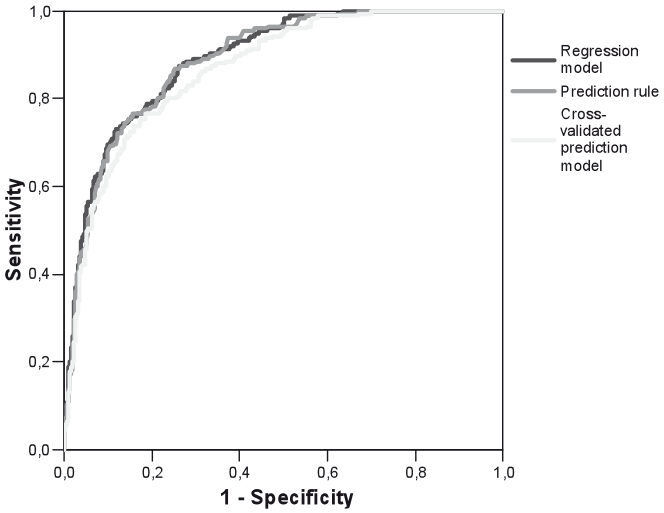

Figure 3. Receiver operating characteristic curve for the logistic regression model, the prediction rule, and the cross-validated prediction model. The area under the curve values for the logistic regression model, the prediction rule, and the cross-validated prediction model were 0.89, 0.89, and 0.87, respectively.

External validation

In the validation cohort, 47% of patients with UA had experienced progression to RA after 1 year of followup. The prediction scores for the patients with UA in whom RA did develop and those in whom RA did not develop are presented in ***Figure 4***. Among patients with UA who experienced progression to RA, the median prediction score was 8.0 (IQR 6.1-9.1); among patients in whom RA did not develop, the median prediction score was 4.6 (IQR 3.5-5.5). Ninety-four percent of the patients with a prediction score ≤6.0 had not experienced progression to RA, and the development of RA was observed in 83% of patients with a score >6. All patients with a score ≥8.0 had progressed to RA, and 78% of patients with a score <8 did not develop RA. In the validation cohort, 17% of the

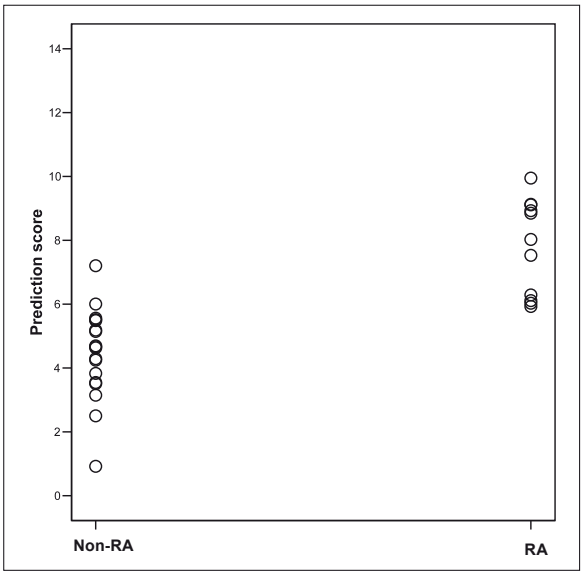

Figure 4. Prediction scores for patients with undifferentiated arthritis in whom rheumatoid arthritis (RA) did develop and those in whom RA did not develop.

patients with UA had a prediction score between 6 and 8; RA had not developed in two-thirds of these patients and had developed in one-third of them. If treatment decisions were based on the prediction rule using cutoff levels of ≥8 for initiating treatment and ≤6 for withholding treatment, treatment would have been withheld inaccurately from only 6% of the patients, and no patient would have received treatment inaccurately. The mean ± SD AUC value for the validation cohort was 0.97 ± 0.024.

Discussion

The currently developed rule predicts the development of RA in patients with UA, using 9 clinical variables that are commonly assessed during the first visit: sex, age, localization of joint symptoms, morning stiffness, tender and swollen joint counts, the CRP level, and the presence of RF and anti-CCP antibodies. The resulting prediction score corresponds to a chance for progression to RA. The positive and negative predictive values of the prediction score depend on the chosen cutoff values. The discriminative ability was excellent, with an AUC value of 0.89 and with a value of 0.87 after internal validation correcting for overfitting. Subsequent validation in a small independent cohort revealed an AUC value of 0.97. Because the prediction rule is accurate and can be easily determined in daily clinical practice, the present model is an important step forward in achieving individualized treatment in patients with recent-onset UA.

Because current evidence regarding treatment of RA is based on results of large trials involving patients fulfilling the ACR 1987 revised criteria for RA (19), fulfillment of these criteria was used as the outcome for the current study. Alternative outcome measurements such as disease persistence or remission can be considered, but no generally accepted definitions of these disease states are available, and no trials of patients with these disease states are available to provide guidance when making treatment decisions. Nevertheless, the use of fulfillment of the ACR criteria as outcome may lead to circularity, because use of the items included in the ACR criteria is expected to result in the identification of predictive variables. However, several studies have shown that the ACR criteria themselves have low discriminative value in patients with UA (12;21-25), and only some of the variables used for the present prediction rule are among the ACR criteria. In the end, it will most likely not make a large difference whether the outcome of a prediction rule is the diagnosis RA or disease persistence, because the ACR criteria are formulated based on patients with longstanding/persistent RA (mean disease duration 8 years), and the reported remission rate in these patients is low (10-15%) (26;27).

Misclassification may have occurred when patients who presented with UA were treated with any drug that hampered the progression to RA. In such cases, patients who normally would have progressed to RA would be classified as non-RA. Exclusion of such misclassified patients, who supposedly would have high prediction scores because they were prone to the development of RA, will result in an increased discriminative ability of the current prediction rule.

The presence of erosions on radiographs of the hands and/or feet is reported to have high specificity (but low sensitivity) for discriminating between self-limiting and persistent disease (25). Although in univariate analysis, the presence of erosions was significantly increased in UA patients in whom RA developed compared with patients in whom RA did not develop (16% versus 7%), multivariate regression analysis revealed that the presence of erosions was not an independent prognostic variable. The presence of erosions appeared to be associated with a higher median age (64 years in patients with erosive disease versus 49 years in those with nonerosive disease), a higher median number of swollen joints (5 joints in patients with erosive disease versus 2 joints in those with nonerosive disease), and the presence of RF (46% of patients with erosive disease versus 23% of those with nonerosive disease). Because the presence of erosions was not identified as a variable with an independent predictive value, data on erosions were not included in the prediction rule.

A model for predicting self-limiting, persisting, or erosive arthritis exists (25). For the development of that model, all consecutive patients with arthritis who were referred were incorporated, including patients in whom a definite diagnosis was made during the first weeks. Decisions regarding the initiation of DMARDs are seldom problematic in such patients. At present, support is needed in making treatment decisions for patients

with recent-onset UA (28), because the disease outcome in patients with UA is variable. For the present study, we selected patients with UA from a total of 1,700 consecutive patients and developed a prediction rule specifically for UA.

The positive and negative predictive values of the prediction score depend on the chosen cutoff level. When the upper and lower cutoff values were 8.0 and 6.0, the corresponding positive predictive value and negative predictive value were 84% and 91%, respectively. In the original cohort, 25% of patients had a prediction score between 6.0 and 8.0; in these patients, the chance of RA developing or not developing was equal. Apparently, the clinical characteristics of patients with intermediate scores are insufficient to predict disease outcome. It is possible that genotype data are helpful in these patients. Patients were also typed for HLA-DRB1 shared epitope (SE) alleles and PTPN22. In the multivariate analysis, the presence of SE alleles was not identified as an independent predictive variable; this might be attributable to the fact that the SE alleles are associated with the presence of anti-CCP antibodies (29), which are already included in the prediction rule. Also, the presence of the PTPN22 T allele did not result in a better ability to predict progression from UA to RA. This is understandable, because the PTPN22 T allele confers risk of both UA and RA (30). In the validation cohort, 17% of patients had a prediction score between 6.0 and 8.0; for treatment decisions in these patients the observed risk of progression to RA can be weighted against the individual risk profile for treatment toxicity.

The prediction score discriminated even better in the validation cohort than in the initial cohort: 100% of patients with a score ≥8.0 had progressed to RA, and 94% of patients with a score ≤6.0 did not develop RA. This indicates that if treatment decisions were based on the prediction rule using the cutoff levels of ≥8 for initiating treatment and ≤6 for withholding treatment, treatment would be inaccurately withheld in only 6% of patients, and no patient would receive treatment inaccurately. Although the validation cohort is relatively small and the current prediction rule should be evaluated in other early arthritis cohorts, we believe that the current model allows physicians and patients to make an evidence-based choice regarding whether or not to initiate DMARDs in the majority of patients presenting with UA.

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CHAPTER 7

Pretreatment serum levels of anti-cyclic citrullinated peptide antibodies are associated with the response to methotrexate in recent-onset arthritis

K. Visser
K.N. Verpoort
H. van Dongen
S.M. van der Kooij
C.F. Allaart
R.E.M. Toes
T.W.J. Huizinga
A.H.M. van der Helm-van Mil

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To direct individual treatment decisions in recent-onset rheumatoid arthritis (RA), predictors of treatment response to methotrexate (MTX) need to be identified. Disease activity at baseline, gender and genetic polymorphisms have already been found to be associated with the effect of MTX treatment, but the predictive value of autoimmune antibody status remains less clear(1;2). It has been shown, however, that both the presence and level of anti-cyclic citrullinated peptide antibodies (ACPA) are strongly associated with a worse disease course (3). Therefore, we investigated the potential predictive effect of levels of ACPA in ACPA-positive patients for the response to MTX treatment. As observations from our cohort and others indicate that ACPA levels decrease during treatment, we studied two selected populations of disease-modifying antirheumatic drug (DMARD)-naïve, ACPA-positive patients with recent-onset arthritis, for whom pretreatment ACPA levels were available (4;5).

All ACPA-positive patients with undifferentiated arthritis (UA) who were included in the PROMPT study (PRObable RA: Methotrexate versus Placebo Treatment) (MTX group n=12, placebo group n=15) were enrolled (5). MTX treatment was started with 15 mg/week and every 3 months the dosage was increased according to the Disease Activity Score (DAS44) to a maximum of 30 mg/week. Responders were defined as patients whose UA did not progress to RA (according to the American College of Rheumatology criteria) during the use of MTX (n=6/12). MTX responders had lower levels of pretreatment IgG ACPA than non-responders (median (interquartile range) 428 (214–643) AU/

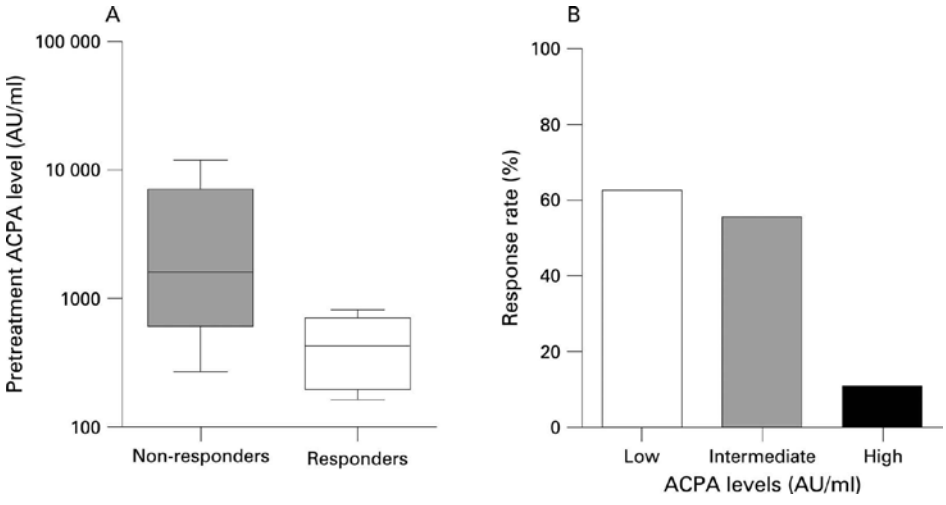

Figure 1. (A) Pretreatment anti-cyclic citrullinated peptide antibodies (ACPA) levels in methotrexate (MTX) responders versus non-responders within ACPA-positive patients with recent-onset, undifferentiated arthritis from the PROMPT study (n=12, p=0.024). (B) Percentage of responders to MTX in ACPA-positive patients with recent-onset rheumatoid arthritis from the BeSt study with low, intermediate and high pretreatment ACPA levels (n=26, p=0.062).

ml vs 1594 (781–4495) AU/ml, respectively) (p=0.024, Mann–Whitney test, ***figure 1A***. Further univariate analysis did not show any significant differences in baseline clinical measures of disease activity, or in rheumatoid factor levels, between responders and non-responders. In addition, the risk of progression to RA, as analysed by survival analysis, was lower in patients with low or intermediate pretreatment ACPA levels than in patients with high levels (stratified by tertiles) (p<0.001, log-rank test, ***figure 2***).

Similar associations were found in a second cohort of ACPA-positive patients with recent-onset RA, who were treated with initial MTX monotherapy (15–25 mg) aiming at a DAS44 ≤2.4 in the BeSt study and from whom pretreatment serum samples were available (n=26/131) (6). Responders were defined as patients achieving a DAS44 ≤2.4 after 6 months. The percentage of responders decreased from 63% and 56% in patients with low and intermediate levels, respectively, to 11% in patients with high levels (stratified by tertiles) (p=0.062, $\chi 2$ test, ***figure 1B***). In a multivariate logistic regression analysis, low and intermediate ACPA levels predicted responsiveness, independently of baseline DAS, gender and age (odds ratio=37, 95% confidence interval 0.8 to 1692, p=0.064).

Despite the limited number of patients, these data from two distinct cohorts suggest that low and intermediate pretreatment levels of ACPA are associated with a more favourable response to MTX treatment in recent-onset, ACPA-positive arthritis, whereas high levels are associated with an insufficient response. Although these findings have to be confirmed in larger studies, quantitative evaluation of ACPA levels might be an additional tool to determine which patients will benefit most from MTX treatment. Therefore, we propose that pretreatment ACPA levels should be used in future prediction analyses.

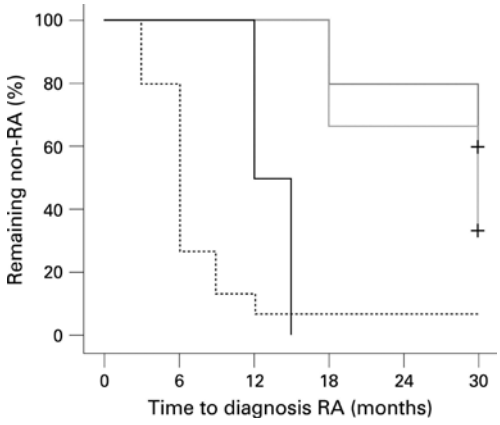

Figure 2. Kaplan–Meier survival curves for the progression of undifferentiated arthritis to rheumatoid arthritis (RA) in anti-cyclic citrullinated peptide antibody (ACPA)-positive patients with undifferentiated arthritis. Placebo group (broken line, n=15); methotrexate group (solid lines, n=12; categorised into patients with low (light line), intermediate (semi-dark line) and high (dark line) pretreatment ACPA levels by tertiles). p<0.001 for the comparison of low/intermediate versus high levels.

Acknowledgements

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CHAPTER 8

Suppressor activity among CD4+, CD25++ T cells is discriminated by membrane-bound tumor necrosis factor alpha

J. Wang
H. van Dongen
H.U. Scherer
T.W.J. Huizinga
R.E.M. Toes

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Abstract

Objective

Previous studies have shown that the suppressive capacity of CD4+,CD25++ T cells is compromised in patients with rheumatoid arthritis (RA) and restored by anti-tumor necrosis factor α(anti-TNFα) therapy. Given the lack of specific cell surface markers for human Treg cells, this study aimed to define surface markers for identifying and enriching Treg cells with enhanced regulatory ability within the CD4+,CD25++ T cell compartment and to provide additional understanding of the effects of anti-TNFα antibodies in humans.

Methods

The expression of membrane-bound TNFα in human peripheral blood CD4+ T cells was analyzed by flow cytometry in healthy individuals and RA patients before and after anti-TNFα treatment. Membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ T cells were purified by fluorescence-activated cell sorting, and their suppressive capacity was assessed in vitro by a standard suppression assay.

Results

A substantial number of CD4+,CD25++ T cells expressed membrane-bound TNFα. Membrane-bound TNFα-positive CD4+,CD25++ T cells displayed reduced antiinflammatory cytokine production and less potent suppressor capacity, since 4 times more cells were required to achieve 50% inhibition compared with their membrane-bound TNFα-negative counterparts. Treatment of RA patients with TNFα-specific antibodies led to a reduction in the number of membrane-bound TNFα-positive CD4+,CD25++ T cells from peripheral blood.

Conclusion

Our data indicate that the absence of membrane-bound TNFα on CD4+,CD25++ T cells can be used to characterize and enrich for Treg cells with maximal suppressor potency. Enrichment of membrane-bound TNFα-negative CD4+,CD25+ cells in the CD4+,CD25++ T cell compartment may contribute to restoring the compromised suppressive ability of CD4+,CD25++ T cell populations in RA patients after anti-TNFα treatment.

Introduction

CD4+,CD25+ Treg cells are generated in the thymus and periphery and represent a crucial regulator of peripheral self-tolerance (1;2). They are hyporesponsive to T cell receptor (TCR) stimulation in vitro (3;4); however, once activated, they can suppress the proliferation and cytokine production of effector T cells, antibody production of B cells, and the function of monocyte/macrophages (3-6). Hence, CD4+,CD25+ Treg cells have been successfully used to prevent/treat immunologic diseases in a variety of animal models, such as collagen-induced arthritis (CIA) (7-9).

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic synovial inflammation resulting in cartilage and bone damage, eventually leading to joint destruction. Several different cell types and their mediators are thought to be involved in this tissue-destructive inflammation, including T cells, B cells, and proinflammatory cytokines such as tumor necrosis factor α(TNFα). The chronic inflammatory process suggests that immune regulation in RA patients is disturbed. Indeed, numerous reports have outlined disturbances in CD4+,CD25+ T cell functions in patients with RA as well as other autoimmune diseases (10-15), suggesting an imbalance between Treg cell and effector T cell activities. However, functional CD4+,CD25+ Treg cells have also been reported in RA patients (16;17), which could be a consequence of the complex phenotype of these cells in humans and the lack of specific cell surface markers to define and isolate Treg cells.

Although CD25 appears to identify a relatively homogeneous population of Treg cells in naive mice, the presence of significant amounts of activated conventional CD25+ T cells in humans makes it difficult to distinguish "genuine" Treg cells from activated conventional T cells in the CD4+,CD25+ T cell compartment. Indeed, several studies have suggested that only the CD4+ T cell subset expressing the highest levels of CD25 (termed $CD25^{high}$ or CD25++) is mostly forkhead box P3-positive (FoxP3+) and has in vitro suppressive activity (18). Nonetheless, activated "conventional" T cells may still be present in the CD4+,CD25++ T cell subset, because FoxP3, the most commonly used marker for Treg cells (19), is also transiently expressed in activated nonsuppressive T cells (20-22). For this reason, the CD4+,CD25++ T cell population that homogeneously expresses FoxP3 might not consist entirely of Treg cells but could also contain conventional activated CD4+,CD25++ T cells, especially in situations in which chronic T cell activation is thought to occur. The possible "contamination" of Treg cells with "conventional" CD4+,CD25++ T cells might also contribute to the compromised suppressive activity of CD4+,CD25++ T cell populations in autoimmune diseases such as RA (10-15), in which chronic T cell activation has been implicated. Likewise, other surface markers, such as CTLA-4 and glucocorticoid-induced TNF receptor, which have been reported to be expressed on Treg cells (23;24), do not distinguish Treg cells from activated effector cells

in humans. Therefore, they cannot be used as such to identify Treg cells, especially not in conditions with chronic T cell activation. Hence, further characterization and therapeutic use of Treg cells would be greatly facilitated by the identification of surface markers that could be used to define and isolate Treg cells with high suppressive activity.

TNFα is a pleiotropic cytokine that is bioactive both as a transmembrane protein and as a homotrimeric secreted molecule (25). Membrane-bound TNFα is expressed on normal human peripheral blood T cells after activation (26). As part of a range of proteins that are expressed on the cell surface after T cell activation, membrane-bound TNFα on CD4+ T cells can modulate the activation of macrophages and costimulate B cells, thereby enhancing their antibody production (27-29). Because it is conceivable that these latter features are mediated by conventional effector T cells rather than by Treg cells, and given that T cells also up-regulate CD25 upon activation, we reasoned that membrane-bound TNFα-expressing CD4+,CD25++ T cells are enriched for activated conventional T cells rather than for naturally occurring Treg cells. In that case, membrane-bound TNFα-positive CD4+,CD25++ T cells would differ in phenotype and/or functional activity compared with their membrane-bound TNFα-negative counterparts.

In the present study, we show that the expression of membrane-bound TNFα on CD4+,CD25++ T cells is correlated with disease activity in RA patients. Although membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ T cells express comparable levels of FoxP3 and are both hyporesponsive to TCR stimulation, membrane-bound TNFα-negative CD4+,CD25++ T cells produce more of the antiinflammatory cytokine interleukin-10 (IL-10) and suppress the proliferation and cytokine production of responder T cells in a more profound manner compared with their membrane-bound TNFα-positive counterparts. Furthermore, treatment with anti-TNFα antibodies results in a reduced frequency of membrane-bound TNFα-positive T cells in the CD4+,CD25++ T cell compartment in RA patients, suggesting that selective depletion of the less suppressive membrane-bound TNFα-positive CD4+,CD25++ T cells by anti-TNFα antibodies contributes to the restoration of Treg cell activity in RA patients that has been reported previously (10-12;30).

Patients and methods

Samples

Fresh peripheral blood was obtained from healthy adult donors with no history of autoimmune diseases. Fresh peripheral blood from RA patients was obtained from patients attending the outpatient clinic of the Leiden University Medical Center. All RA patients fulfilled the 1987 revised classification criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (31). The characteristics of these

RA patients are summarized in ***Table 1***. Buffy coats were obtained from the Sanquin Bloodbank (Sanquin, Amsterdam, The Netherlands). Informed consent was provided in accordance with procedures approved by the local human ethics committee.

Table 1. Characteristics of and disease parameters for the RA patients*

Age, years	55.2 ± 14.5
RA duration, years	5.55 ± 2.79
DAS†	1.60 ± 0.88
ESR, mm/hour†	17.6 ± 15.6
No. of women/no. of men	22/18
No. RF positive/no. RF negative‡	22/5
No. anti-CCP-positive/no. anti-CCP negative‡	18/9

* Except where indicated otherwise, values are the mean ± SEM. RA = rheumatoid arthritis.
† Data on the Disease Activity Score (DAS) and erythrocyte sedimentation rate (ESR) were available for 23 patients when membrane-bound tumor necrosis factor α(TNFα) expression was analyzed.
‡ Data on rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) status were available for 27 patients when membrane-bound TNFα expression was analyzed.

Antibodies

Fluorescein isothiocyanate (FITC)-conjugated anti-CD25 (2A3), phycoerythrin (PE)-conjugated anti-CD25 (M-A251), anti-CD127 (hIL-7R-M21), anti-CD45RO (UCHL1), allophycocyanin (APC)-conjugated anti-CD4 (SK3), anti-CD25 (M-A251), and peridin chlorophyll protein-Cy5.5-conjugated anti-CD4 (SK3) were all from BD Biosciences (San Jose, CA). PE/FITC-coupled anti-FoxP3 (PCH101; eBioscience, San Diego, CA) was used as indicated. Anti-TNFα monoclonal antibody (infliximab) was labeled with FITC (Calbiochem, La Jolla, CA) and used to detect the expression of membrane-bound TNFα on cells.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood or buffy coats by centrifugation over Ficoll-Hypaque gradients. CD4+ T cells were enriched from PBMCs by negative selection with the CD4+ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). CD4+ T cells were then stained with APC-conjugated anti-CD4, PE-conjugated anti-CD25, and FITC-conjugated anti-TNFα for 30 minutes at 4°C. After washing, CD4+,CD25- responder T cells, membrane-bound TNFα-positive cells, and membrane-bound TNFα-negative cells within the CD4+,CD25++ T cell population were isolated by sorting with an Aria flow sorting machine (BD Biosciences). The sorted cells were gated first on small lymphocytes by forward and side scatter, then for CD4+ and CD25++ (top 2-5% of CD4+ T cells) expression, and then for membrane-bound TNFα expression. CD4-depleted PBMCs irradiated with 4,500 rads were used as feeders.

Flow cytometric analysis

Single-cell suspensions were prepared, and surface molecules were stained for 30 minutes at 4°C with optimal dilutions of each antibody. After fixation and permeabilization, cells were incubated with anti-FoxP3 antibody. Expression of cell surface and intracellular markers was assessed using flow cytometry (FACSCalibur; Becton Dickinson, Mountain View, CA) after gating on live cells determined by scatter characteristics. Data were analyzed by CellQuest Pro software (Becton Dickinson).

Proliferation and suppression of T cells

Cells were plated at 1 × 104 cells/well in 96-well plates with 1 µg/ml phytohemagglutinin (PHA) and feeders (5 × 104 cells/well) in the presence or absence of 50 units/ml IL-2. Cells were pulsed with 3H-thymidine (0.5 µCi/well) on day 4, and proliferation was assessed 18 hours later using a liquid scintillation counter. To test for suppressive capacity, CD4+,CD25- responder T cells were stimulated as described above without the addition of exogenous IL-2. Autologous membrane-bound TNFα-positive CD4+,CD25++ T cells or membrane-bound TNFα-negative CD4+,CD25++ T cells were added, and suppression was assessed by determining 3H-thymidine incorporation as well as by measuring the amounts of TNFα and interferon-γ (IFNγ) in culture supernatants.

Cytokine detection

To determine the concentration of cytokines, BD CBA Flex Sets (for IL-10 and TNFα) (BD Biosciences) or a capture enzyme-linked immunosorbent assay (for IFNγ) was used to analyze culture supernatants after 96 hours, following the protocol provided by the manufacturer. The beads were analyzed on an LSRII system using BD FCAP Array software (BD Biosciences).

Statistical analysis

The Mann-Whitney U test was used to compare Treg cell-mediated suppression results and cell frequencies in healthy controls and RA patients. Paired t-tests were used to compare measurements in RA patients before and after anti-TNFα treatment. Spearman's correlations were used to compare cell frequencies with subject age. All statistical analyses were performed using GraphPad Prism 4.00 software (GraphPad Software, San Diego, CA). *P* values less than 0.05 were considered significant.

Results

Preferential expression of membrane-bound TNFα on CD4+,CD25++ T cells in vivo

Recent findings have indicated that the suppressive function of CD4+,CD25++ T cells is compromised in patients with active RA (10;11). However, the suppressive capability of the CD4+,CD25++ T cell population is reversed after anti-TNFα therapy (10-12;30). Given the prominent role of TNFα in inflammation and in inflammatory autoimmune diseases such as RA (32-34), and given the effector functions of membrane-bound TNFα on activated CD4+ T cells (i.e., macrophage activation and increment of antibody production) (27-29), it is conceivable that the membrane-bound TNFα-expressing CD4+,CD25++ T cell population is enriched for "conventional" activated T cells.

We therefore hypothesized that this T cell population possesses inferior suppressive abilities compared with the non-membrane-bound TNFα-expressing CD4+,CD25++ T cell population. Furthermore, we speculated that membrane-bound TNFα-positive CD4+,CD25++ T cells are preferentially depleted following anti-TNFα therapy, which in turn could contribute, at least partially, to the reappearance of the suppressive function of isolated CD4+,CD25++ T cell populations from RA patients after anti-TNFα therapy (10-12;30). Hence, we wished to examine the phenotype and function of membrane-bound TNFα-expressing cells in more detail to determine whether these characteristics could be used to distinguish T cells with different suppressive potency within the CD4+,CD25++ T cell compartment. To this end, we first characterized membrane-bound TNFα expression on freshly isolated human CD4+ T cells.

PBMCs from RA patients were purified, and membrane-bound TNFα expression was detected by flow cytometry. Similar proportions of CD4+,CD25- (mean 13.9% [range 3.43-30.8) and CD4+, $CD25^{intermediate}$ (mean 13.3% [range 3.68-28.1]) T cells stained positive for membrane-bound TNFα. However, within the CD4+,CD25++ T cell compartment, a significantly higher percentage of membrane-bound TNFα-positive T cells (mean 26.6% [range 7.49-49.2]) was observed ***(Figures 1A and B)***. Similar results were obtained with cells isolated from healthy individuals (n = 19) (data not shown). Furthermore, a consistent increase in the percentage of membrane-bound TNFα-positive T cells was observed within the FoxP3+,CD4+ T cell population compared with their FoxP3-,CD4+ counterparts (data not shown). These results could not be explained by binding of the antibody to TNFα captured by surface TNF receptors (TNFRs), because most TNFRII+,CD4+,CD25++ cells are membrane-bound TNFα negative, and, more important, in line with previous reports (35), similar membrane-bound TNFα expression was observed after preincubation of the cells with high concentrations of soluble TNFα (10 and 50 ng/ml) (data not shown). Together, these data indicate that human CD4+,CD25++ T cells express membrane-bound TNFα.

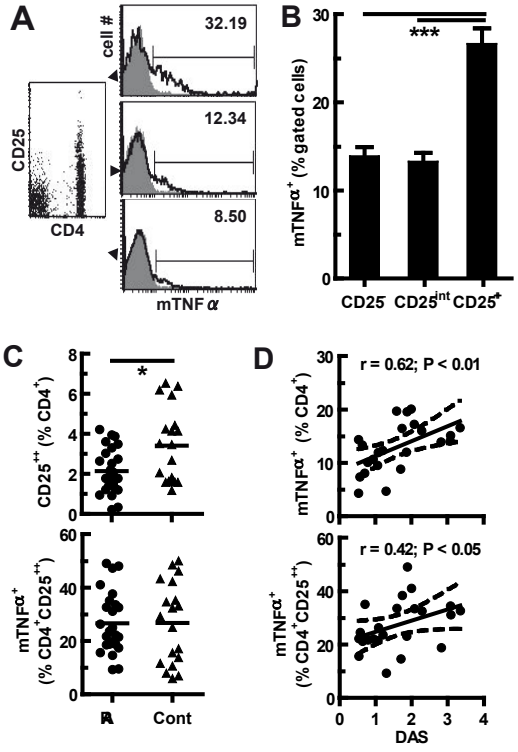

Figure 1. Correlation of membrane-bound tumor necrosis factor α(mTNFα) expression with disease activity in patients with rheumatoid arthritis (RA). Peripheral blood mononuclear cells were isolated from RA patients and stained with anti-CD4, anti-CD25, and anti-TNFα (infliximab). CD25 and membrane-bound TNFα expression was monitored by flow cytometry. All patients analyzed were naive for TNFα antagonists. A, Representative histograms (right panel) showing membrane-bound TNFα staining (black line) and isotype staining (shaded area) within each cell population. The gates for CD4+,CD25- (bottom box), CD4+,CD25^{intermediate} (CD4+,CD25^{int}; middle box), and CD4+,CD25++ (top box) T cells are demonstrated in the left panel. Numbers within boxes in the right panel indicate the percentage of positive cells within each gate. B, Bar graph of the percentage of membrane-bound TNFα-expressing cells in different T cell populations. Results are expressed as the mean and SEM from 40 different individuals. **= $P < 0.001$. C, Comparison of CD4+,CD25++ or membrane-bound TNFα-expressing CD4+,CD25++ T cells between 26 RA patients (mean ± SEM age 46.7 ± 10.9 years) and 19 age-matched healthy controls (Cont) (mean ± SEM age 44.3 ± 13.8 years). Horizontal lines indicate median values for each group. *= $P < 0.05$. D, Frequency of membrane-bound TNFα-expressing cells within CD4+ and CD4+,CD25++ T cell compartments, plotted against the Disease Activity Scores (DAS) for 23 patients. Linear regression (solid line) with 95% confidence intervals (dashed lines) are shown.

Correlation of membrane-bound TNFα expression with disease activity in RA patients

We next wished to study whether the expression of membrane-bound TNFα on CD4+,CD25++ T cells differs between healthy controls and RA patients. To this end, we compared CD25 and membrane-bound TNFα expression in 26 RA patients with that in

age-matched healthy controls. Although the number of CD4+,CD25++ T cells decreased significantly in these RA patients, there was no significant difference in the frequency of membrane-bound TNFα-expressing CD4+,CD25++ T cells ***(Figure 1C)***.

The Disease Activity Score (DAS) (36) was available for 23 of the 40 RA patients studied at the same time that membrane-bound TNFα expression was analyzed ***(Table 1)***. Given the role of TNFα in the pathogenesis of RA (32-34) and the expression and function of membrane-bound TNFα on activated CD4+ T cells (25-29), we wished to address the question of whether there is any association between disease activity and the expression of membrane-bound TNFα. As shown in ***Figure 1D***, the analysis revealed that the percentages of membrane-bound TNFα-positive T cells within the CD4+ ($r = 0.62$, $P < 0.01$) or CD4+,CD25++ ($r = 0.42$, $P < 0.05$) T cell populations were correlated with disease activity in these RA patients. Given that membrane-bound TNFα expression was also associated with the erythrocyte sedimentation rate in RA patients (data not shown), these data indicated that the expression of membrane-bound TNFα is associated with the inflammatory immune response in RA patients. No association of its expression with disease duration was observed (data not shown).

Expression of other markers on membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ T cells

Given the association of membrane-bound TNFα-expressing CD4+,CD25++ T cells with disease activity (Figure 1D) and the possibly compromised suppressive activity of CD4+,CD25++ T cells in patients with active RA, as previously reported (10;11), we wished to characterize these membrane-bound TNFα-expressing CD4+,CD25++ T cells in more detail. Since FoxP3 is the most commonly used marker for human CD4+,CD25+ Treg cells thus far (19), and because surface CD127 expression is inversely associated with the presence of FoxP3 and the suppressive function of freshly isolated human CD4+ T cells from healthy donors (37), we first determined how surface membrane-bound TNFα expression correlates with FoxP3 and CD127 expression. Therefore, PBMCs were stained for CD4, CD25, and membrane-bound TNFα, together with either FoxP3 or CD127.

As shown in ***Figure 2***, no significant difference in intracellular FoxP3 expression was observed between membrane-bound TNFα-positive and membrane-bound TNFα-negative CD4+,CD25++ T cells, either in the percentage of positive cells or in the expression levels within each cell compartment. Likewise, a similar percentage of CD127-/^{low} cells was observed within the membrane-bound TNFα-negative CD4+,CD25++ and membrane-bound TNFα-positive CD4+,CD25++ T cell subsets. Because the expression of CD45RO, the memory marker for human T cells, has been used to discriminate different CD4+,CD25+ Treg cell populations (38), we analyzed the expression of this marker on membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ T cells as well. Almost all membrane-bound TNFα-positive CD4+,CD25++ T cells displayed

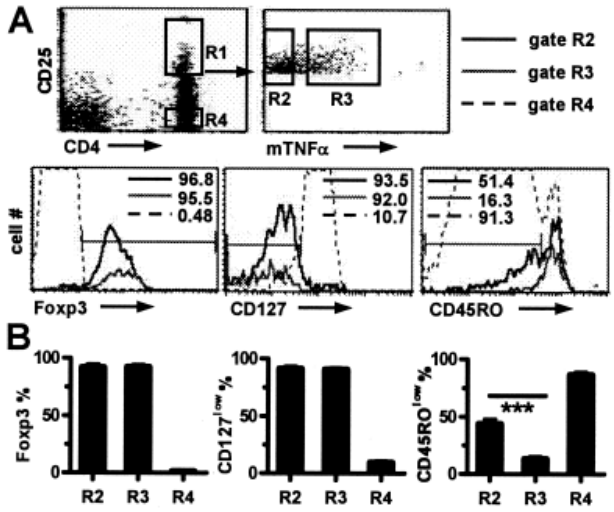

Figure 2. Expression of other markers on membrane-bound tumor necrosis factor α(mTNFα)-positive and mTNFα-negative CD4+,CD25++ T cells. Peripheral blood mononuclear cells were stained with anti-CD4, anti-CD25, and anti-TNFα, together with anti-CD127, anti-CD45RO, or anti-forkhead box P3 (anti-FoxP3) (intracellular for FoxP3). A, Gating for histograms was performed on the basis of CD4, CD25, and membrane-bound TNFα expression, as indicated in the upper panel. Membrane-bound TNFα-negative CD4+,CD25++ (region 2 [R2]) and membrane-bound TNFα-positive CD4+,CD25++ (R3) T cell subsets were gated by first restricting to CD4+,CD25++ T cells (R1). Percentages of FoxP3+, CD127-/^{low}, and CD45RO-/^{low} cells within each gate are shown in the lower panel. CD4+,CD25- T cells (R4) were included as a control. B, Bar graph shows the percentages of FoxP3+, CD127-/^{low}, and CD45RO-/^{low} cells within each gate as shown in A. Results are expressed as the mean and SEM from 8 different individuals. ***= $P < 0.001$.

high expression of CD45RO, whereas a significant proportion of their membrane-bound TNFα-negative counterparts had lower levels of CD45RO expression (Figure 2). Similar results were obtained when membrane-bound TNFα-positive or TNFα-negative CD4+,CD25+ T cells were analyzed (data not shown). Together, these data indicate that membrane-bound TNFα expression on CD4+,CD25++ T cells correlates with levels of CD45RO expression, despite similar FoxP3 and CD127 expression.

Association of membrane-bound TNFα with decreased production of antiinflammatory cytokines in CD4+,CD25++ T cells

Human CD4+,CD25++ Treg cells are hyporesponsive and produce relatively high levels of the antiinflammatory cytokine IL-10 but low levels of proinflammatory cytokines such as TNFα and IFNγ (39). To analyze whether membrane-bound TNFα expression on CD4+,CD25++ T cells discriminates cells with differential cytokine production profiles, we purified membrane-bound TNFα-positive CD4+,CD25++ and membrane-bound TNFα-negative CD4+,CD25++ T cells from buffy coats using fluorescence-activated cell

sorting (FACS), and we subsequently stimulated them with PHA and autologous feeders in the presence or absence of exogenous IL-2.

With respect to proliferation and production of the proinflammatory cytokines TNFα and IFNγ, both membrane-bound TNFα-positive and membrane-bound TNFα-negative

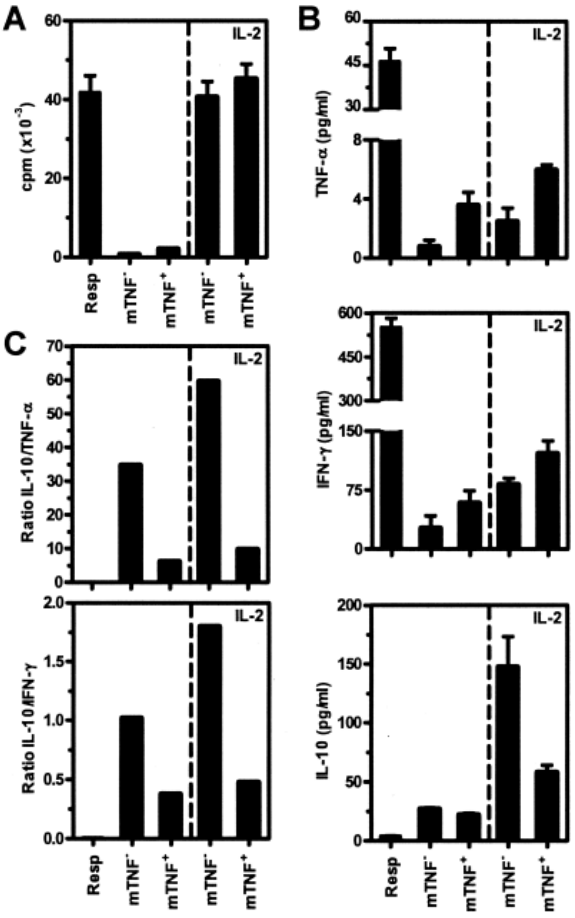

Figure 3. Association of membrane-bound tumor necrosis factor α(mTNFα) with decreased production of antiinflammatory cytokines in CD4+,CD25++ T cells. Fluorescence-activated cell-sorted CD4+,CD25- responder T cells (Resp), membrane-bound TNFα-negative CD4+,CD25++ T cells (mTNF-), and membrane-bound TNFα-positive CD4+,CD25++ T cells (mTNF+) (1 × 104 cells/well) were activated with phytohemagglutinin (1 μg/ml) and feeders (5 × 104 cells/well) in the absence or presence of 50 units/ml exogenous interleukin-2 (IL-2). The sorting gates for membrane-bound TNFα-negative and membrane-bound TNFα-positive CD4+,CD25++ T cells are depicted in the upper panel of Figure 2A. A, Cell proliferation was determined by 3H-thymidine uptake 5 days later. B and C, Culture supernatants were collected after 96 hours, and the amounts of TNFα, interferon-γ (IFNγ), and IL-10 produced (B) or the ratios of IL-10:TNFα and IL-10:IFNγ (C) were determined. Results are expressed as the mean and SEM from 3 or 4 triplicate cultures and are representative of those from 4 independent experiments.

CD4+,CD25++ T cells were hyporesponsive compared with autologous CD4+,CD25- responder T cells ***(Figures 3A and B)***. Nonetheless, when membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ cells were compared side by side, membrane-bound TNFα-positive CD4+,CD25++ T cells produced more TNFα and IFNγ (Figure 3B) and displayed a higher rate of proliferation (Figure 3A). In contrast, both CD4+,CD25++ T cell populations produced more IL-10 upon stimulation compared with CD4+,CD25- T cells (Figure 3B). Intriguingly, however, membrane-bound TNFα-negative CD4+,CD25++ T cells produced twice the amount of IL-10 in comparison with their membrane-bound TNFα-positive counterparts after stimulation in the presence of IL-2 (Figure 3B). The mean ± SEM IL-10 level produced by membrane-bound TNFα-negative CD4+,CD25++ T cells was 121.99 ± 14.27 pg/ml compared with 51.64 ± 7.68 pg/ml produced by their membrane-bound TNFα-positive counterparts after 4 days of stimulation in the presence of IL-2 ($P < 0.05$; $n = 4$) (data not shown).

These results indicate a difference in functional capacity between these two CD4+,CD25++ T cell subsets, which is also illustrated by the ratio of IL-10 to TNFα or IFNγ ***(Figure 3C)***. Together, these data point to the possibility that membrane-bound TNFα-negative CD4+,CD25++ T cells display a greater suppressive potency compared with their membrane-bound TNFα-expressing counterparts.

Membrane-bound TNFα on CD4+,CD25++ T cells distinguishes a population with enhanced suppressor activity

To address whether differential membrane-bound TNFα expression is indeed associated with differential suppressive activity, fluorescence-activated cell-sorted membrane-bound TNFα-positive CD4+,CD25++ and membrane-bound TNFα-negative CD4+,CD25++ T cells were placed in a standard in vitro suppression assay using CD4+,CD25- T cells as responder T cells. As shown in ***Figure 4A***, membrane-bound TNFα-negative CD4+,CD25++ T cells suppressed proliferation more robustly than did membrane-bound TNFα-positive CD4+,CD25++ T cells. Approximately 4-fold fewer membrane-bound TNFα-negative CD4+,CD25++ cells than membrane-bound TNFα-positive CD4+,CD25++ cells were required to achieve 50% suppression ***(Figure 4B)***.

To further confirm the differential suppressive capability between membrane-bound TNFα-positive and TNFα-negative CD4+,CD25++ cells, we next analyzed the amounts of TNFα and IFNγ in the coculture supernatants. Consistent with the results described above, the addition of membrane-bound TNFα- negative CD4+,CD25++ T cells at a 1:1 ratio resulted in a stronger reduction of TNFα and IFNγ production than the addition of membrane-bound TNFα-positive CD4+,CD25++ T cells ***(Figure 4C)***. This difference could not be attributed to the higher cytokine production by membrane-bound TNFα-positive CD4+,CD25++ T cells, since they secreted relatively small amounts of TNFα and IFNγ under these conditions (Figure 3B). Taken together, these observations indicate that

membrane-bound TNFα-negative CD4+,CD25++ T cells possess an enhanced suppressive activity.

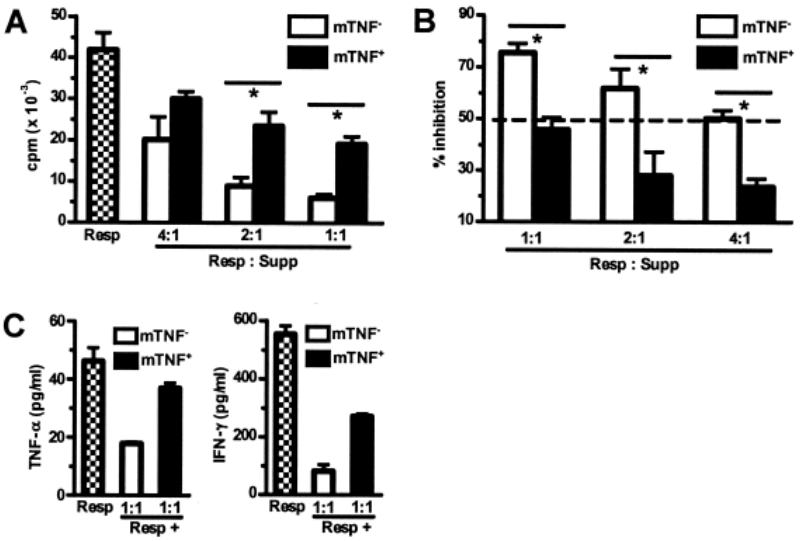

Figure 4. Membrane-bound tumor necrosis factor α(mTNFα) on CD4+,CD25++ T cells distinguishes a population with enhanced suppressor activity. Fluorescence-activated cell-sorted CD4+,CD25- responder T cells (Resp) (1 × 104 cells/well) were activated with phytohemagglutinin (1 µg/ml) and feeders (5 × 104 cells/well) in the absence or presence of membrane-bound TNFα-negative CD4+,CD25++ suppressor T cells (Supp) or membrane-bound TNFα-positive CD4+,CD25++ suppressor T cells (mTNF- or mTNF+, respectively) at a 4:1, 2:1, or 1:1 (Resp:Supp) ratio. In all experiments, CD4+,CD25- responder T cells were also added at a 1:1 ratio as a control (i.e., 2 × 104 responder T cells per well), which always yielded greater proliferation and cytokine production (data not shown). A, Proliferation after 5 days was determined by ^{3}H-thymidine incorporation. B, The average percent inhibition of proliferation is shown. The dashed line indicates 50% inhibition. C, Culture supernatants were collected after 96 hours, and the amounts of TNFα and interferon-γ (IFNγ) were analyzed in cultures of responders only (Resp) or responders plus suppressors (Resp +) at a 1:1 ratio. Results are expressed as the mean and SEM from 3 or 4 cultures and represent (A and C) or summarize (B) 4 independent experiments with different individuals. *= $P < 0.05$.

Decreased numbers of membrane-bound TNFα-positive T cells in RA patients after anti-TNFα treatment

Previous studies have shown that anti-TNFα therapy could reverse the compromised function of isolated CD4+,CD25++ T cells in RA patients (10-12;30). Given our observations that membrane-bound TNFα-positive CD4+,CD25++ T cells bear a less potent suppressor activity (Figure 4) and are associated with disease activity in RA patients (Figure 1D), we wished to know whether the expression of membrane-bound TNFα on T cells was decreased in RA patients after TNFα-blocking therapy (adalimumab; n = 7).

All these patients responded well to anti-TNFα treatment, as evidenced by a reduction in the mean ± SEM DAS in 28 joints (40), from 7.06 ± 0.56 to 3.89 ± 0.66 three months

after treatment ($P < 0.0001$) (data not shown). There was no difference in the absolute number and the frequency of CD3+ T cells, CD4+ T cells, and CD4+,CD25++ T cells in peripheral blood (***Figures 5A and B*** and data not shown) before and 3 months after adalimumab treatment. However, a significantly decreased expression of membrane-bound TNFα on CD4+,CD25++ T cells (Figures 5A and B) as well as on total CD3+ or CD4+ T cells (data not shown) was observed in all patients. This could not be explained by the presence of adalimumab on membrane-bound TNFα-positive T cells in vivo that would influence the detection of membrane-bound TNFα expression ex vivo by infliximab, since the same expression levels of membrane-bound TNFα were observed on cells regardless of whether or not they had been preincubated with adalimumab ***(Figure 5C)***.

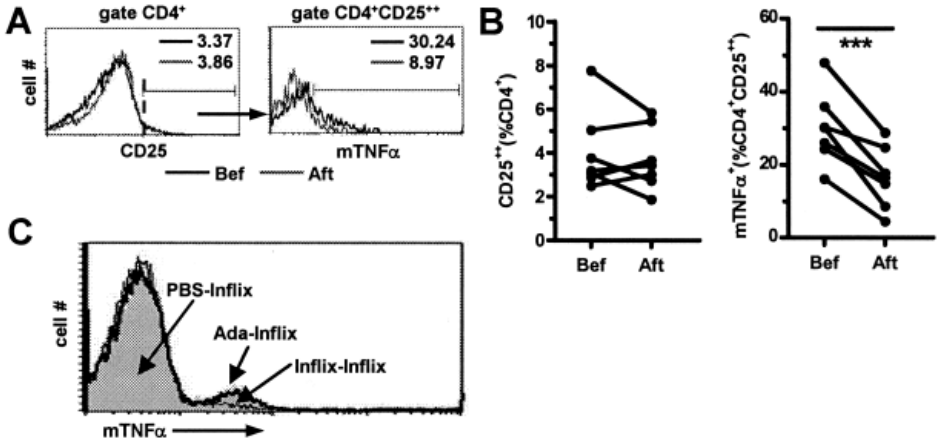

Figure 5. Decreased expression of membrane-bound tumor necrosis factor α(mTNFα) in patients with rheumatoid arthritis (RA) after anti-TNFα treatment. Seven patients with active RA (Disease Activity Score in 28 joints >5) were evaluated for CD25 and membrane-bound TNFα expression both before and 3 months after anti-TNFα therapy (40 mg adalimumab subcutaneously every other week in combination with stable doses of methotrexate [7.5-25 mg/week orally]). A, Representative histograms showing CD25 staining on CD4+ T cells or membrane-bound TNFα expression on CD4+,CD25++ T cells in RA patients before (Bef) and 3 months after (Aft) adalimumab treatment. Numbers indicate the percentage of CD25++ expression on CD4+ T cells (left panel) or membrane-bound TNFα expression on CD44+,CD25++ T cells (right panel). B, Summary of the percentage of CD25++ T cells within CD4+ T cells or of the frequency of CD4+,CD25++ T cells coexpressing membrane-bound TNFα in RA patients before and 3 months after TNFα-blocking therapy. Each dot represents 1 patient (n = 7). C, Histogram showing lack of effect of adalimumab on the detection of membrane-bound TNFα by fluorescein isothiocyanate (FITC)-labeled infliximab. Cells were incubated with phosphate buffered saline (PBS-Inflix; shaded area), adalimumab (Ada-Inflix; thick black line), or infliximab (Inflix-Inflix; thin black line) at 4°C for 30 minutes. After washing, cells were stained for membrane-bound TNFα expression by FITC-labeled infliximab. ***= $P < 0.001$.

Discussion

Immunosuppressive CD4+,CD25++ Treg cells are of great interest for immunotherapy to prevent transplant rejection and for the treatment of autoimmune diseases. Successful treatment of mice with CIA, an animal model for human RA, by adoptive transfer of CD4+,CD25+ Treg cells has been reported (9). However, unlike the situation in mice, in which expression of CD25 identifies a relatively homogeneous population of Treg cells, the identification and manipulation of Treg cells in humans is hampered due to the presence of large amounts of activated conventional T cells within the CD4+,CD25+ T cell compartment. Although the highest expression of CD25 (termed $CD25^{high}$ or CD25++) on CD4+ T cells is now widely used to identify, isolate, and characterize naturally occurring Treg cells in humans, the reported defective suppressive function of the isolated CD4+,CD25++ T cell population from patients with autoimmune diseases, such as RA, might still be attributed, at least in part, to the heterogeneous composition of this T cell population (10-15). Therefore, one of the main obstacles to therapeutic applications of Treg cells is to define and isolate cells displaying high inhibitory activity.

TNFα is a pleiotropic cytokine critical for inflammation, maintenance of secondary lymphoid organ structure, and host defense against various pathogens as well as for the pathogenesis of RA (32-34;41;42). Moreover, membrane-bound TNFα expressed on the surface by CD4+ T cells activates macrophages, costimulates B cell activation, and increases antibody production (27-29). Therefore, we speculated that membrane-bound TNFα-expressing CD4+,CD25++ T cells are enriched for activated conventional T cells and/or T cells with inferior suppressive function, since Treg cells inhibit the function of monocyte/macrophages, reduce antibody production of B cells, and produce very small amounts of TNFα, even in the presence of IL-2 (5;6;21). Our results reveal a significant expression of membrane-bound TNFα within the CD4+,CD25++ T cell population both in RA patients and in healthy individuals (Figure 1 and data not shown). Although membrane-bound TNFα-positive CD4+,CD25++ T cells display suppressive activity, this activity is much less potent than that of their membrane-bound TNFα-negative counterparts (Figure 4). Therefore, we conclude that membrane-bound TNFα expression is a marker that is inversely associated with the suppressive capacity of CD4+,CD25++ T cells.

It has been shown that the surface expression of CD127 is inversely correlated with the expression of FoxP3 and the suppressive function of fresh human CD4+ T cells (37). However, our data indicate that these 2 markers are not differentially expressed by CD4+,CD25++ T cells that do and those that do not express membrane-bound TNFα(Figure 2). Although FoxP3 was thought to program the development and function of murine CD4+,CD25+ Treg cells (19), FoxP3 is not a Treg cell-specific marker in humans, since it is also expressed in activated effector T cells (20-22;43). Likewise, not all of the

CD4+,CD127-/^{low} cells in human PBMCs are FoxP3+, since CD127 is down-regulated in recently activated effector T cells (22;37). Therefore, our data suggest that the expression levels of FoxP3 or CD127 do not correlate with the suppressive activity *within* the CD4+,CD25++ T cell compartment, whereas the absence of membrane- bound TNFα on CD4+,CD25++ T cells can be used to characterize and enrich Treg cells with maximal suppressor potency.

Recent reports have suggested that in vivo a portion of the human CD4+,CD25++ T cell population is generated from rapidly dividing, highly differentiated memory CD4+ T cells (44;45). Since the memory T cell marker CD45RO is highly expressed on membrane-bound TNFα-positive CD4+,CD25++ T cells (Figure 2), and membrane-bound TNFα expression on these cells correlates with disease activity (Figure 1D), it is tempting to speculate that these FoxP3+, anergic membrane-bound TNFα-positive CD4+,CD25++ T cells are "adaptive" Treg cells derived from memory T cells in the periphery. Such cells could emerge as a negative feedback to dampen immune responses, as proposed previously (44-47). Our results showing that a subset of CD4+,CD25++ T cells display a less potent suppressive capacity and bear additional activation markers, such as CD45RO, would support this notion. The reason why such cells also express membrane-bound TNFα could relate to their former function as effector cells, during which they secreted TNFα. The expression of membrane-bound TNFα would, in that context, be a residual property of these cells. This could also explain the correlation between their presence and disease activity, since it is conceivable that greater disease activity would lead to more exhausted effector T cells that express membrane-bound TNFα.

Treatment of RA patients with TNFα blockers results in significant clinical benefit, which most likely involves the inhibition of the TNFα-induced inflammatory cytokine cascades (34). Moreover, recent studies have suggested that anti-TNFα antibodies could also reverse the "compromised" suppressive function of isolated CD4+,CD25++ T cells in RA patients (10-12;30). This restoration is thought to involve the neutralization of circulating soluble TNFα that inhibits Treg cell function via TNFRII and/or the induction of a distinct CD62L-,CD25++ Treg cell population via transforming growth factor βin RA patients (11;12). Considering the fact that adalimumab could deplete membrane-bound TNFα-expressing cells via inducing apoptosis, antibody-dependent cellular cytotoxicity, and/or complement-dependent cytotoxicity both in vitro and in vivo (48-50), our results provide an additional explanation for the reversal of Treg cell activity of the isolated CD4+,CD25++ T cell population after anti-TNFα antibody therapy, since they indicate that these antibodies deplete the less suppressive membrane-bound TNFα-positive CD4+,CD25++ T cells from the CD4+,CD25++ T cell population in RA patients (Figures 4 and 5).

In conclusion, we have demonstrated that membrane-bound TNFα is expressed on a significant percentage of CD4+,CD25++ T cells in human peripheral blood, and that this

expression is correlated with disease activity in RA patients. Moreover, the absence of surface membrane-bound TNFα expression can be used to identify and isolate a subset of CD4+,CD25++ T cells with potent suppressive capability. Furthermore, our results indicate that, in addition to blocking soluble circulating TNFα and/or inducing a new Treg cell population (11;12), selective depletion of the less effective membrane-bound TNFα-positive suppressors from the CD4+,CD25++ T cell compartment in RA patients may be another explanation for the recovery of Treg cell function by anti-TNFα therapy.

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CHAPTER 9

Functional regulatory immune responses against human cartilage glycoprotein-39 in health vs. proinflammatory responses in rheumatoid arthritis

J.H.M. van Bilsen
H. van Dongen
L.R.Lard
E.I.H van der Voort
D.G. Elferink
A.M. Bakker
A.M.M. Miltenburg
T.W.J. Huizinga
R.R.P. de Vries
R.E.M. Toes

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Abstract

The class of immune response against autoantigens could profoundly influence the onset and/or outcome of autoimmune diseases. Until now, there is only limited information on the antigen-specific balance between proinflammatory and regulatory responses in humans. Here we analyzed the natural immune response against a candidate autoantigen in rheumatoid arthritis, human cartilage glycoprotein-39 (HC gp-39). Peripheral blood mononuclear cells from healthy individuals reacted against HC gp-39 with the production of IL-10 but not IFN-γ. Ex vivo assays indicated that the naturally occurring HC gp-39-specific immune response in bulk is powerful enough to suppress antigen-specific recall responses, demonstrating that rather than being unresponsive, the HC gp-39-directed immune response in healthy individuals shows a strong bias toward a regulatory phenotype. Moreover, CD4+ T cell lines directed against HC gp-39 expressed CD25, glucocorticoid-induced tumor necrosis factor receptor, and Foxp3 molecules and were capable of suppressing antigen-specific T cell responses. Cell–cell contact was required for this suppression. As opposed to healthy individuals, the HC gp-39-directed immune response in 50% of patients with rheumatoid arthritis exhibits polarization toward a proinflammatory T helper 1 phenotype and is significantly less powerful in suppressing antigen-specific recall responses. Together these findings indicate that the presence of HC gp-39-specific immune responses in healthy individuals may have an inhibitory effect on inflammatory responses in areas where HC gp-39 is present. Furthermore, these data indicate that the class of HC gp-39-directed immune response in rheumatoid arthritis patients has shifted from an antiinflammatory toward a proinflammatory phenotype.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease with a variable disease outcome, which is characterized by an inflammatory process of unknown origin in multiple joints. Although the pathogenesis of RA is multifactorial, RA susceptibility and/or severity is strongly associated with the presence of isotype-switched autoantibodies and certain HLA class II alleles. These observations clearly point to a role for T cells in the pathological processes underlying RA. Therefore, much interest has focused on the phenotype of the T cell response that is present in the inflamed joints of RA patients. In general, these T cells display a typical T helper (Th)1-like phenotype (1). These T cells, either directly or indirectly, contribute to the proinflammatory cascades and inflammatory environment that is observed within the joints of RA patients (2). In contrast, other mechanisms are likely to be involved in the protection against autoimmunity such as the production of antiinflammatory cytokines such as IL-10 and TGF-β. In this respect, a population of naturally occurring T cells, T regulatory (Treg) cells, have regained much interest in the last decade. These cells display antiinflammatory and antiproliferative functions. In several animal model systems, it has been shown that Treg cells play a critical role in the generation and maintenance of tolerance and generally inhibit immune responses that are potentially deleterious to the host (3). Several T cell subsets have been described that mediate these protective immunoregulatory effects, of which the most extensively studied T cell subset is characterized by the expression of CD4 and CD25 in both mice and humans (4–6).

Investigations into the role of Treg cells have been difficult because no specific marker for Treg cells has been identified so far. The expression of CD25, cytotoxic T lymphocyte antigen-4, the tumor necrosis factor (TNF) family molecule glucocorticoid-induced TNF receptor (GITR), and Foxp3 have been used to identify Treg cells but, in general, Treg cells are characterized on the basis of a functional definition through their ability to down-regulate other immune responses (7, 8).

In vitro studies indicate that T cell receptor triggering by, for example, anti-CD3 is required for Treg cell function (3, 9), indicating that antigen recognition most likely represents the natural trigger initiating Treg cell activity. However, there is only limited evidence for autoantigen-specific regulatory T cell populations in humans. Nonetheless, knowledge on the autoantigens capable of inducing regulatory circuits in humans is important because this knowledge can be used for the design of rationally defined vaccines that prevent and/or inhibit autoimmune diseases. Studying the question whether a certain antigen can induce regulatory circuits in humans has been proven difficult because of various technical aspects related to low precursor frequencies and the inherent difficulty of expanding cells with a regulatory phenotype *in vitro*. Moreover, the choice of the antigenic stimulus is particularly important and, in case a certain antigen

is selected for further studies, high purity to avoid immune activation by contaminants, such as lipopolysaccharide, within the antigen preparation is essential.

Here, we have studied the natural immune responses against human cartilage glycoprotein-39 (HC gp-39), a candidate autoantigen in RA. HC gp-39 is a major constituent of human cartilage and is overexpressed in synovial specimens and cartilage from RA patients (10). It has been shown that HC gp-39 is efficiently processed and presented in the context of MHC class II molecules by dendritic cells (DCs) and macrophages (11, 12). HC gp-39 can also serve as a target for T cells, because HC gp-39-specific MHC class-II-restricted T cell responses can readily be induced in HLA-DR4-transgenic mice (13). Moreover, proliferative responses have been described in RA patients, indicating that HC gp-39-directed T cells are present also in humans (13–15). Interestingly, intranasal treatment with HC gp-39 suppressed murine collagen-induced arthritis (16). These observations indicate that HC gp-39 can activate/boost regulatory circuits that interfere with arthritis in case the antigen is presented in an environment that is adapted to induce tolerogenic responses to innocuous antigens. Moreover, they indicate that HC gp-39-directed T cells can escape thymic deletion and that HC gp-39-specific T cells are present in the natural T cell repertoire. Together, these observations identify HC gp-39 as an interesting human autoantigen and have led us to hypothesize that HC gp-39 is driving regulatory responses in humans. Therefore, the aim of this work was to determine whether HC gp-39 could stimulate regulatory or suppressive immune responses in healthy individuals and whether the bias of HC gp-39-directed immunity is altered in RA patients.

Materials and methods

Blood Samples and HLA-DR Typing

After informed consent, citrated or heparinized venous blood was collected from healthy blood bank donors and RA patients classified as having definite RA according to the 1987 American College of Rheumatology criteria for RA (17). The characteristics of the RA patients are summarized in ***Table 1***. Peripheral blood mononuclear cells (PBMCs) were isolated and used directly or were cryopreserved in liquid nitrogen. HLA-DR typing was performed on genomic DNA by using the sequence specific oligonucleotide (PCR-SSO) as described in refs. 18 and 19. In this study, DRB1*04-positive donors were selected for further analysis.

Antigens

Clinical-grade human recombinant HC gp-39 (Org 39141; 10 µg/ml final conc.) was kindly provided by N.V. Organon, Oss, The Netherlands. The recall-response antigen

Table 1. Characteristics of RA patients

No of patients tested	43
Age, years	53.7 ± 13.9 (24.0-78.5)*
Male/female	15/28
HLA-DR4	43/43
Disease duration, years	6.8 ± 1.8 (3.3-9.7)*
Erosive disease	34/43 (81%)
RF positive	33/43 (76.7%)
DMARD treatment	40/43 (93%)
More than one DMARD in use	11/43 (25.6)
More than one DMARD used in the past	30/43 (69.8%)
Methotrexate treatment	23/43 (53.5%)
Prednisolone treatment	3/47 (7.0%)

RF, rheumatoid factor-IgM (RF postitive if ≥5 units; DMARD, disease modifying antirheumatic drug.
*Expressed as mean ± SD (range, in parentheses).

mix consisted of a mixture of tetanus toxoid (T.tox) (0.75 limit of flocculation unit/ml final conc.; National Institute of Public Health and the Environment, Bilthoven, The Netherlands), purified protein derivative (PPD) from *Mycobacterium tuberculosis* (5 µg/ml final conc.; Statens Serum Institute Copenhagen), and *Candida albicans* (0.005%, HAL Allergenen Lab, Haarlem, The Netherlands).

Keyhole limpet hemocyanin (KLH; 10 µg/ml final conc.) was obtained from Pierce Biotechnology. Phytohaemagglutinin (PHA HA16; 2 µg/ml final conc.) was obtained from Remel (Lenexa, KS). Clinical-grade human insulin (Actrapid; 10 µg/ml final conc.) was obtained from Novo Nordisk, Bagsvaerd, Denmark. The preferentially expressed antigen of melanoma (PRAME)-derived peptide $PRA^{142–151}$ (SLYSFPEPEA; 5 ng/ml final conc.) was synthesized by solid-phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422, Langenfeld, Germany) and subsequently stored at –20°C until usage.

Cytokine Elispot Analysis

PBMCs were stimulated with antigen in culture medium supplemented with 10% FCS for 4 days in 24-well plates. Thereafter, the cells were seeded in 4 replicate wells at a density of 10^5 cells per well (IFN-γ) or 1.5×10^5 cells per well (IL-10) of a MultiScreen 96-well plate (Millipore), coated with IFN-γ or IL-10 catching antibody (MABTECH, Natcha, Sweden). After overnight incubation at 37°C, the plates were developed according to the manufacturer's procedures. The number of visible spots was counted in a BioReader 3000 (BioSys, Karben, Germany). To examine the nature of the responder cells, PBMCs were cultured as described above or depleted of CD3+ T cells (>97% pure). The CD3-negative fraction was seeded at a density of 5×10^4 cells per well. For statistical analysis of differences between cocultures in the inhibition assays, Student's *t* test was used.

Differences in IFN-γ production between healthy donors and RA patients were analyzed by the Mann–Whitney *U* test.

Detection of Antigen-Specific IL-10-Secreting Cells

Fresh PBMCs were stimulated with antigen in culture medium supplemented with 10% autologous or AB-serum for 4 days in 24-well plates. Thereafter, the cells were labeled with anti-IL-10 antibody (Miltenyi Biotec, Amsterdam) and subsequently seeded overnight in six-well plates. Next, the IL-10 secreting cells were stained according to the manufacturer's procedures and analyzed on a FACSCalibur (Becton Dickinson). Isotype-matched mouse IgG1-phycoerythrin-conjugated control mAb (clone X40) was obtained from Becton Dickinson.

Generation of T Cell Lines

PBMCs were stimulated *in vitro* (2×10^6 per ml) with PPD (5 μg/ml), T.tox. (0.75 limit of flocculation unit/ml) or HC gp-39 (10 μg/ml). After 4–5 days, cells were expanded for 11–13 days in culture medium supplemented with 10% FCS and recombinant human IL-2 (rhIL-2; 25 units/ml; Chiron) alone [T cell line raised against PPD from *Mycobacterium tuberculosis* (T_{PPD})/T cell line raised against T.tox. ($T_{T.tox}$)] or rhIL-2 combined with rhIL-15 (10 units/ml; TEBU BIO Peprotech, Heerhugowaard, The Netherlands) for the T cell line raised against HC gp-39 ($T_{HCgp\text{-}39}$).

Proliferation Assays

Proliferation of T cell lines was measured in 96-well plates in 4–6 replicate cultures. To analyze putative toxic properties of HC gp-39, 2.5×10^3 cells from T_{PPD} or $T_{T.tox}$ were incubated per well together with PPD or T.tox and 5×10^3 irradiated (3,000 rad) autologous PBMCs as antigen-presenting cells (APCs) with or without HC gp-39. No inhibition of proliferation was detected in T_{PPD} or $T_{T.tox}$, indicating that HC gp-39 was nontoxic. For the inhibition assays, each well contained 10^4 T_{PPD} cells, 10^4 autologous $T_{HCgp\text{-}39}$ cells, 2×10^4 irradiated autologous PBMCs as APCs, and antigen. Control antigen clinical grade insulin (Actrapid) was heat pretreated to disrupt 3D structures and prevent putative cell interactions. After 4 days of culturing, proliferation was determined by overnight incorporation of [^{3}H]thymidine. For statistical analysis the two-tailed Student's *t* test for unpaired samples was used.

Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE)-Labeled Cell Division Analysis

T_{PPD} or $T_{T.tox}$ were labeled with 0.5 μM CFSE (Molecular Probes). The CFSE-labeled cells (5×10^5) were incubated with 10^6 irradiated PBMCs as APCs, antigen, and with or without 5×10^5 unlabeled cells from an autologous $T_{HCgp\text{-}39}$. After 3 days, the CFSE content of the T_{PPD}

was analyzed on a FACSCalibur. To quantify the absolute number of divided CFSE-labeled cells and to correct for apoptotic or dead CFSE-labeled cells, the number of analyzed cells was standardized by the addition of 15×10^3 Flow-Count fluorospheres per sample (Beckman Coulter). Acquisition was stopped when 3×10^3 beads were counted.

In the transwell experiments, $T_{HCgp\text{-}39}$ (5×10^5) or control cell line $T_{T.tox}$ were cultured in the inner wells (24-well plate) in medium containing 5×10^5 irradiated PBMCs as APCs with or without HC gp-39 protein. Equal numbers of CFSE-labeled T_{PPD} cells and APCs were added into the outer wells in the same medium with or without PPD. After 4 days of culture, the CFSE content of the T_{PPD} was analyzed on a FACSCalibur.

Recall Suppression Assays

PBMCs were incubated with or without HC gp-39 in a 24-well plate. After 3–5 h, the cultures were supplemented with a recall-response antigen mix (see above). After 4 days of culturing, the overnight IFN-γ excretion of the culture was analyzed by elispot (see above). Differences in the HC gp-39-induced changes in IFN-γ excretion between healthy donors and RA patients were analyzed by the Mann–Whitney *U* test.

Cytotoxic T Lymphocyte (CTL) Inhibition Assays

An HLA-A2-restricted CTL clone directed against a tumor-associated antigen PRAME-derived peptide was a kind gift from J. Kessler (Leiden University Medical Center). The isolation, maintenance, and properties of the CTL clone are described in ref. 20. During stimulation with the PRAME peptide, this CTL clone produces high amounts of IFN-γ. For inhibition assays, PBMCs from an HLA-A2-positive donor known to secrete IL-10 after incubation with HC gp-39 were incubated with or without HC gp-39 protein. After 48 h, the PRAME peptide together with 30,000 A2-restricted PRAME-specific CTLs was added. The overnight IFN-γ excretion of the coculture was analyzed by elispot (see above).

Results

HC gp-39-Specific Immune Reactivity Is Characterized by IL-10 Production

Because various observations indicate the presence of HC gp-39-directed T cells in the human T cell repertoire, combined with the finding that presentation of HC gp-39 in a noninflammatory/tolerogenic environment can induce regulatory circuits (16), we wished to evaluate the natural HC gp-39-directed immune response in healthy individuals. As elispot analyses allow the detection of low precursor frequencies with preservation of the class of immune responsiveness (e.g., as measured by IFN-γ or IL-10 production), we used elispot analyses after a short, 4-day culture of PBMCs with antigens. Our data show that the PBMCs from 17 of 31 healthy donors analyzed reacted against HC

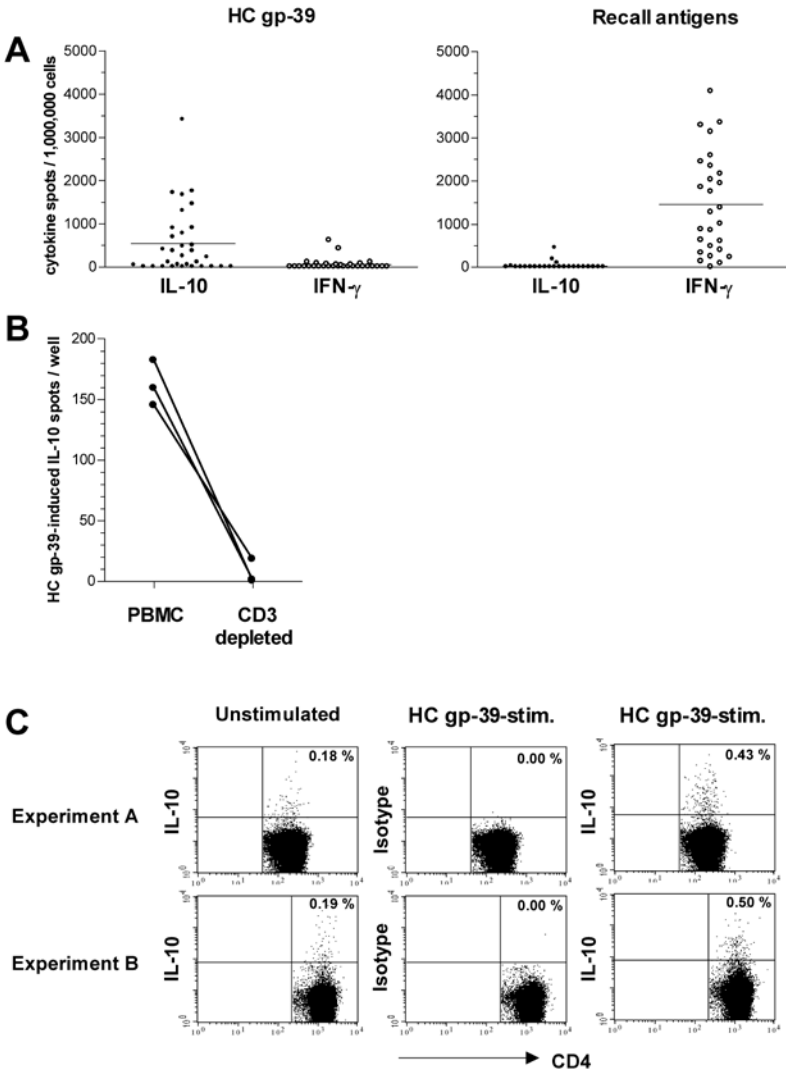

Figure. 1. HC gp-39-specific immune reactivity in healthy donors. (A) PBMCs from healthy donors (n = 31) react against HC gp-39 by production of IL-10 but not IFN-α (Left). Responses induced by "control" recall antigens are dominated by IFN-γ production (Right). (B) HC gp-39-induced IL-10 responses are mediated by T cells. Shown in an analysis of PBMCs from three different donors for IL-10 responses to HC gp-39, in which HC gp-39 was cultured with unmanipulated PBMCs or PBMCs immunomagnetically depleted of CD3+ T cells before elispot analysis. Background responses (medium alone) were subtracted fromHCgp-39 responses. (C) CD4+ T cells respond to HC gp-39 with the production of IL-10. Unstimulated CD4+ T cells (Left) produce background amounts of IL-10 (0.2%), whereas IL-10 was produced by 0.4–0.5% of HC gp-39-stimulated CD4+ T cells (Right). As a control, HC gp-39-stimulated CD4+ T cells (Center) were stained with an isotype-matched control mAb (0.0% staining).

gp-39 with the production of IL-10 without the concomitant production of IFN-γ ***(Figure 1A Left)***. This response is specific for HC gp-39 because responses toward a mix of typical Th1-associated recall antigens are dominated by IFN-γ production ***(Figure 1A Right)***. Importantly, HC gp-39 was not toxic to the cells, because relatively high doses of HC gp-39 did not suppress the proliferative capacity of defined T cell lines (data not shown). No IL-10 responses could be detected anymore after depletion of CD3+ cells from the PBMC cultures stimulated with HC gp-39 ***(Figure 1B)***. In contrast, IL-10 responses remained in CD3-depleted cultures stimulated with phytohemag-glutinin. Together, these findings indicate that T cells or CD3+ natural killer cells are responsible for the IL-10 production after stimulation of PBMCs with HC gp-39. To further validate the source of IL-10 production after HC gp-39-stimulation, we investigated whether CD4+ cells produce IL-10 in response to HC gp-39 by flow cytometry. Although we were not able to detect IL-10 production toward HC gp-39 in most donors by this technique (presumably as a result of a lower sensitivity of this assay compared with elispot analyses), we reproducibly detected CD4+ IL-10+ cells in a donor harboring a high frequency of cells reacting against HC gp-39. As shown in ***Figure 1C***, IL-10 was produced by 0.4–0.5% of CD4+ cells (Right), whereas unstimulated PBMCs produced only background amounts of IL-10 (0.2%) (Left). Together, these results indicate that the natural immune response against HC gp-39 in healthy donors is hallmarked by the production of IL-10 mediated by HC gp-39-directed T cells.

Phenotypic and Immunological Characterization of CD4+ T Cell Lines Against HC gp-39

The observation that CD3+ T cells produce IL-10 and not IFN-γ on exposure to HC gp-39 prompted us to investigate whether HC gp-39-responsive T cells represent T cells with immunoregulatory properties. For this purpose, we generated T cell lines against HC gp-39 ($T_{HCgp\text{-}39}$). $T_{HCgp\text{-}39}$ were expanded by HC gp-39-specific stimulation in the presence of IL-2 and IL-15 (21, 22) and subsequently analyzed for the presence of various markers that are associated with CD4+ T_{reg} cells. Phenotypic analyses of the generated $T_{HCgp\text{-}39}$ showed that $T_{HCgp\text{-}39}$ were CD4+ expressing high levels of CD25 and glucocorticoid-induced tumor necrosis factor receptor that produced increased levels of IL-10, tumor necrosis factor α, and IFN-γ on stimulation with HC gp-39 (data not shown). Moreover, real-time PCR revealed that the $T_{HCgp\text{-}39}$ also expressed relatively high levels of Foxp3, a transcription factor highly expressed in CD4+ CD25+ T_{reg} cells (23, 24). Control Th1 cell lines against PPD and T.tox. that were derived from the same donors displayed similar markers, although the level of expression was often considerably lower (data not shown). These results indicate that the phenotype of the T cell lines against HC gp-39 is compatible with the molecular makeup typically found on naturally occurring CD4+ CD25+ T_{reg} cells.

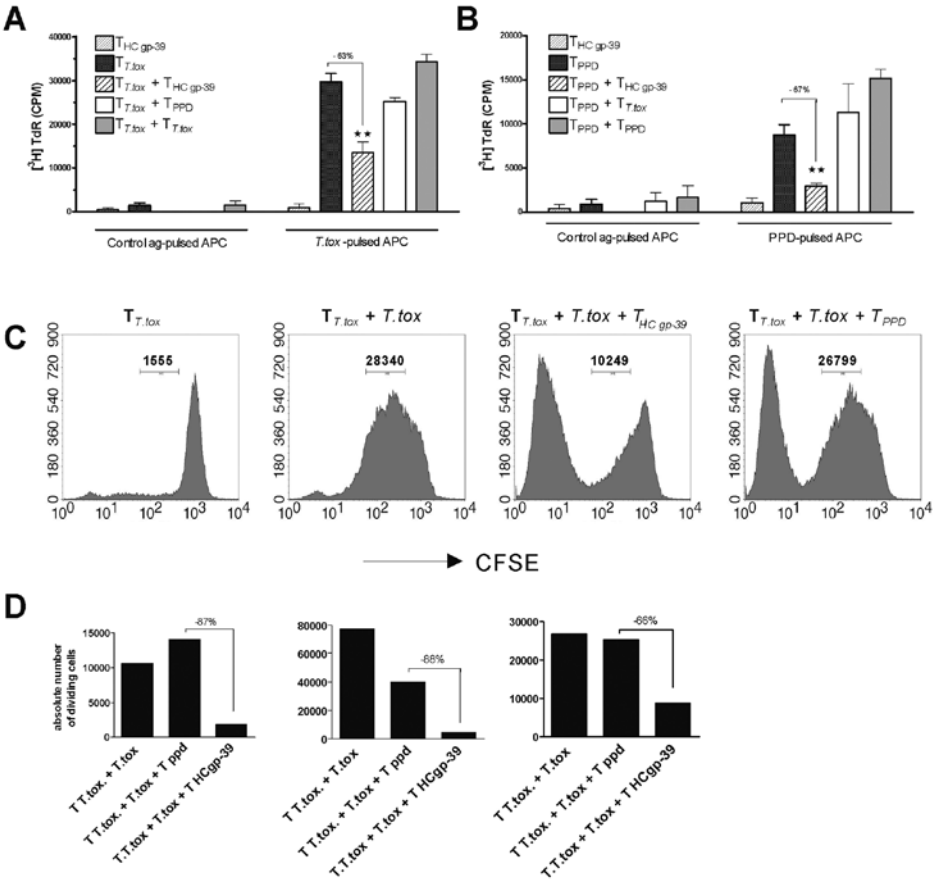

Figure 2. CD4+T cell lines raised against HC gp-39 ($T_{HCgp-39}$) are suppressive. (A and B) $T_{HCgp-39}$ suppresses proliferation of autologous T cell lines specific for recall antigens, $T_{T.tox}$ (A) and T_{PPD} (B). Background responses (unstimulated $T_{T.tox}$ and T_{PPD}) were subtracted from stimulated $T_{T.tox}$ and T_{PPD}. **, $P < 0.01$. (C) $T_{HCgp-39}$ can inhibit cell division of autologous CFSE-labeled $T_{T.tox}$. $T_{T.tox}$ (Far Left) was incubated with T.tox-pulsed APCs alone (Center Left) or together with $T_{HCgp-39}$ (Center Right) or T_{PPD} (Far Right). The absolute number of dividing cells is indicated in each histogram. Similar results were obtained in four donors. (D) $T_{HCgp-39}$ can inhibit proliferation of autologous $T_{T.tox}$ and T_{PPD}. T cell lines directed against T.tox, PPD, and HC gp-39 were generated from three different donors (Left, Center, and Right). Although some suppression of $T_{T.tox}$ proliferation was sometimes observed after addition of T_{PPD} (center bar in Center), a pronounced suppressive activity is displayed by $T_{HCgp-39}$. These data are representative of nine donors analyzed. Similar data were obtained when T_{PPD} was used as a readout, adding $T_{T.tox}$ and $T_{HCgp-39}$ as suppressors (data not shown). Background responses (unstimulated $T_{T.tox}$ and T_{PPD}) were subtracted from T.tox and PPD-stimulated $T_{T.tox}$ and T_{PPD}, respectively.

Nonetheless, more definitive evidence of CD4+ T_{reg} cells resides in the demonstration that these cell lines can inhibit proliferation of conventional CD4+ T cells in functional assays. Therefore, we set out to determine the regulatory capacities of HC gp-39-directed T cells in suppression assays. To this end, $T_{HCgp-39}$ cells were titrated into cultures consisting

of T_{PPD} or $T_{T.tox}$. The proliferative responses of T.tox-activated $T_{T.tox}$ cell lines ***(Figure 2A)*** and PPD-activated T_{PPD} cell lines ***(Figure 2B)*** were suppressed ($P < 0.01$) by the addition of $T_{HCgp-39}$ by up to 67%. In contrast, addition of T.tox-directed T cells to PPD-directed T cells and vice versa did not result in inhibition of proliferation, indicating that the suppression observed after adding $T_{HCgp-39}$ was not because of competition for space or nutrients at high cell concentrations. Moreover, inhibition required the presence of $T_{HCgp-39}$ cells because APCs pulsed with the HC gp-39 protein were not able to mediate suppression (data not shown). HC gp-39-directed T cell lines did not display detectable proliferative responses toward HC gp-39 (data not shown), which is in line with their regulatory phenotype. Together, these findings indicate that HC gp-39 by itself is not able to polarize the APCs into a "regulatory" APC and, more importantly, indicate that HC gp-39-directed T cell lines display regulatory functions.

The suppressive capacity of HC gp-39-directed T cell lines was confirmed in a read-out system where CFSE-labeled T.tox or PPD-directed T cell lines were cocultured with autologous HC gp-39-directed T cells. In this manner, the inhibition of proliferation of antigen-specific T cell lines can be monitored directly in a quantitative manner. ***Figure 2C*** shows that addition of $T_{HCgp-39}$ results in the inhibition of $T_{T.tox\ cell}$ division by 62% (from 28×10^3 to 10×10^3 divided cells), whereas no such inhibition was observed by adding the "crowding" control T_{PPD}. In ***Figure 2D***, the results of three independent experiments are shown employing T cell lines obtained from three different donors. Although we also observed inhibition of the proliferative response after addition of an irrelevant control cell line in one of three cases, the addition of $T_{HCgp-39}$ resulted in a considerably stronger inhibition.

The observation that $T_{HCgp-39}$ displays a regulatory phenotype led us to investigate whether cell–cell contact was required for HC gp-39-directed T cell lines to inhibit other CD4+ effector T cells. The suppressive action of $T_{HCgp-39}$ on CFSE-labeled cell division was abrogated when cell–cell contact between $T_{HCgp-39}$ and CFSE-labeled $T_{T.tox}$ or T_{PPD} was prevented in transwell assays ***(Figure 3 A–D)***. As the control, a simultaneously performed coculture assay was performed in which strong suppression of the proliferative activity of CSFE-labeled T.tox- or PPD-directed T cell lines was observed ***(Figure 3 E and F)***, indicating that the $T_{HCgp-39}$ cells were viable and immunosuppressive. Together these results indicate that $T_{HCgp-39\ cells}$ are able to mediate suppression in a cell–cell contact-dependent fashion.

Ex Vivo HC gp-39-Directed Immune Responses Can Suppress Recall Responses and CD8+ T Cell Activity

As described above, our *in vitro* assays indicate that $T_{HCgp-39}$, once activated, down-regulate other immune responses in a cell–cell contact-dependent manner. To address the question of whether the HC gp-39-reactive immune response directly after cells are

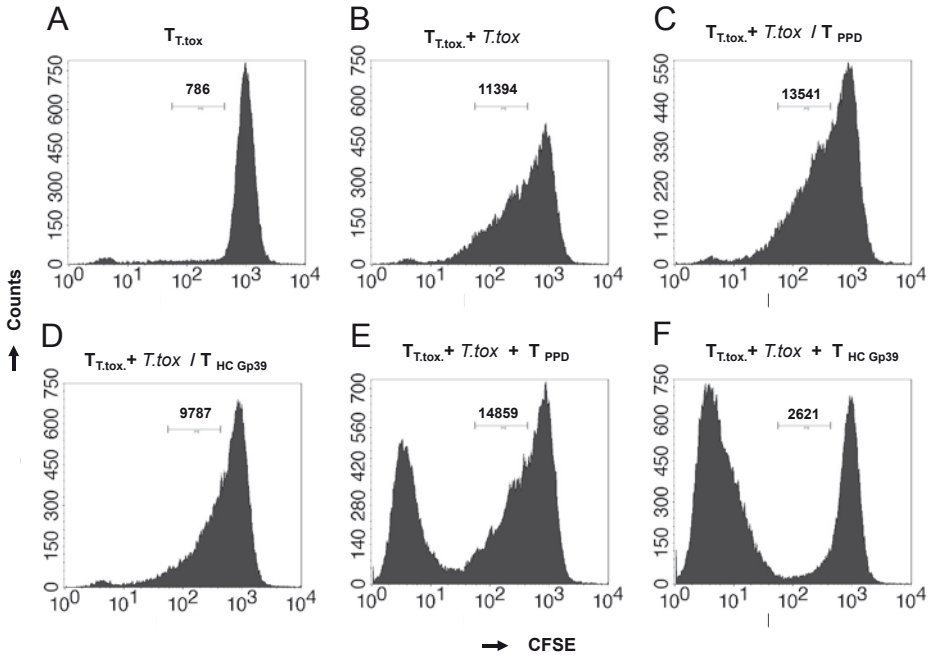

Figure 3. Cell– cell contact is required for the immunosuppressive activity of CD4+T cell lines directed against HC gp-39. The absolute number of dividing CFSE-labeled $T_{T.tox}$ cells is indicated in each histogram. (A) Unstimulated $T_{T.tox}$ in outer wells (B) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells. (C) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells and TPPD in inner wells. (D) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells and $T_{HCgp-39}$ in inner wells. (E) $T_{T.tox}$ stimulated with T.tox-pulsed APCs and T_{PPD} together in outer wells. (F) $T_{T.tox}$ stimulated with T.tox-pulsed APCs and $T_{HCgp-39}$ together in outer wells.

taken from the donors is efficient enough to suppress other immune responses, donors that displayed IL-10 responses after stimulation with HC gp-39 were selected for further study. PBMCs from these donors were stimulated with a 10× diluted mixture of recall antigens to induce intermediate recall antigen-specific IFN-γ responses as measured by elispot analyses. To study whether stimulation by HC gp-39 can suppress this IFN-γ production, we triggered the PBMCs simultaneously with HC gp-39 to activate regulatory cells and the recall antigen mixture to activate recall antigen-specific memory cells. HC gp-39 could stimulate regulatory circuits as observed by a marked inhibition of the recall response ***(Figure 4A)***.

Next, we determined whether HC gp-39 stimulation could also inhibit CD8+ T cell responsiveness. Therefore, an HLA-A2-restricted CTL clone directed against a peptide derived from the tumor antigen PRAME was used. PBMCs from HLA-A2+ donors were stimulated with HC gp-39 and, after 48 h, loaded with the PRAME peptide. Subsequently, the PRAME-specific CTL clone was added, and IFN-γ production was measured by elispot analyses. HC gp-39-stimulated HLA-A2+ PBMCs were able to suppress IFN-γ production

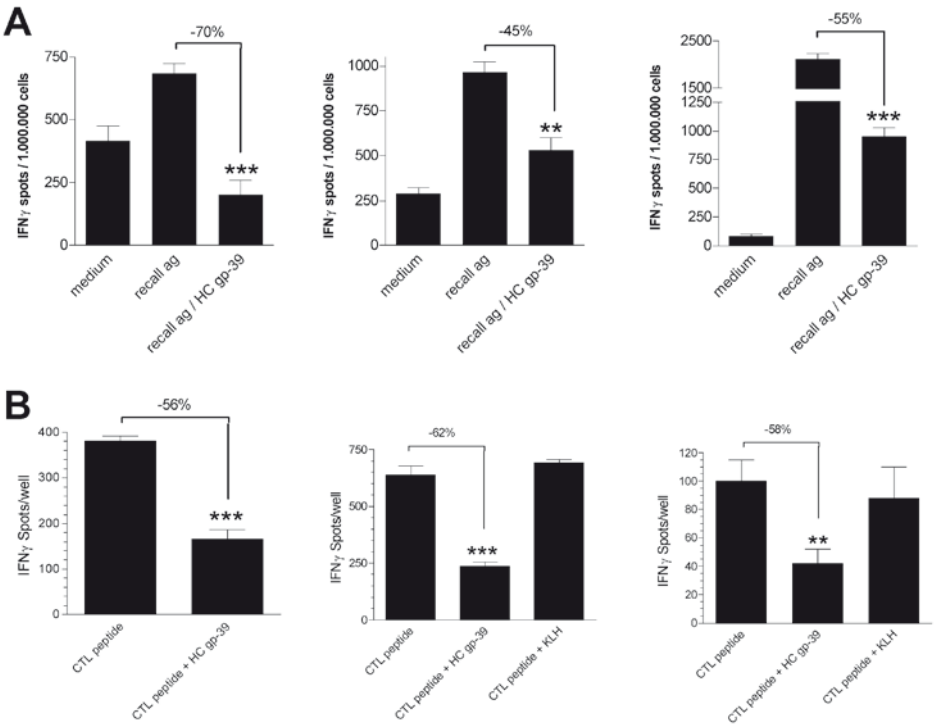

Figure 4. *Ex vivo* HC gp-39-directed immune responses can suppress other immune responses. (A) HC gp-39-activated PBMCs are able to suppress IFN-γ responses to recall antigens in three different healthy donors. (B) HC gp-39- stimulated HLA-A2+ PBMCs from three different healthy donors are capable of suppressing IFN-γ production of activated HLA-A2+ CTLs. **, $P < 0.005$; ***, $P < 0.0005$.

of activated PRAME-specific CTL as shown in ***Figure 4B***. Together these data indicate that the "naturally" occurring HC gp-39-reactive immune response is highly effective in suppressing other immune responses.

The HC gp-39-Directed Immune Response in RA Patients Is Biased Toward a Proinflammatory Response

Intrigued by the antiinflammatory properties of HC gp-39-directed immune responses in healthy donors, we postulated that the immune response in RA patients might have shifted toward a proinflammatory response. Therefore, the immune response in RA patients was evaluated by employing IFN-γ and IL-10 elispots. In contrast to healthy donors, which produce primarily IL-10 ***(Figs. 1 A and 5 A and B)***, RA patients produce considerable amounts of IFN-γ ($P < 0.05$; i.e., 17 of 34 patients analyzed produced IFN-γ). These findings could not be attributed to a generalized increase in IFN-γ production in RA patients because the recall antigen-specific immune reactivity is diminished in RA patients compared with controls ***(Figure 5 C and D)***. Next we compared PBMC cultures

from RA patients (n = 40) with the ability of PBMC cultures from healthy donors (n = 21) to reduce recall antigen responses after costimulation with HC gp-39. It was found that HC gp-39 was less effective in stimulating regulatory response in PBMC cultures from RA patients compared with cultures from healthy controls ***(Figure 5E)*** because the majority of PBMC cultures from RA patients gave rise to more IFN-γ spots after costimulation with HC gp-39. In some patients, this increase was even 4-fold or higher. Together these data imply that a disease-associated bias is displayed by the HC gp-39-directed immune response.

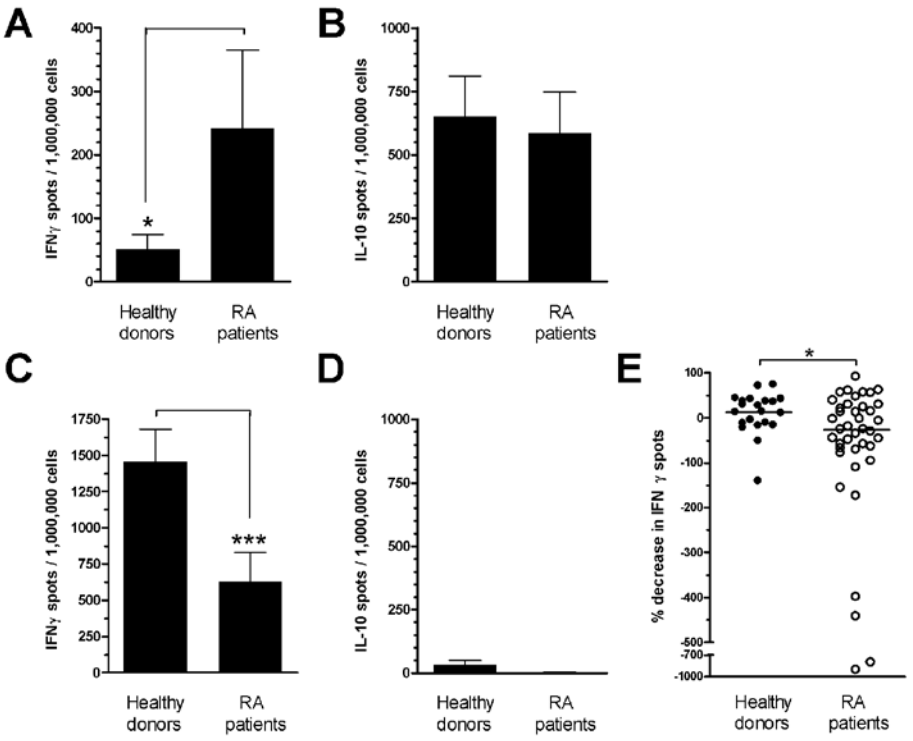

Figure 5. IFN-γ and IL-10 responses to HC gp-39 and recall antigens by PBMCs from healthy donors (n = 31) and RA patients (n = 34) as determined by elispot. (A and B) Healthy donors produce primarily IL-10 but not IFN-γ, whereas RA patients produce both IL-10 and considerable amounts of IFN-γ in response to HC gp-39 (*, $P < 0.05$). (C and D) Recall antigen-directed reactivity is characterized by IFN-γ production but not IL-10 production in both RA patients and healthy donors. Moreover, PBMCs from healthy donors produce more IFN-γ after exposure to recall antigens as compared with RA patients (***, $P < 0.001$). (E) HC gp-39-stimulated PBMCs from healthy donors (n = 21; unbiased for HC gp-39-induced IL-10 production) are better suppressors of recall antigen-induced IFN-γ production than HC gp-39-stimulated PBMCs from RA patients (n = 40). *, $P < 0.03$. Line represents the median in percent decrease per group.

These findings indicate that HC gp-39 serves as an autoantigen in both healthy donors and RA patients. However, despite a reduced Th1 response against recall antigens, the HC gp-39-directed immune responsiveness in RA patients is shifted toward a proinflammatory phenotype in a substantial number of RA patients as more IFN-γ is produced and is paralleled by a reduced suppression of recall responses.

Discussion

In the present study, we demonstrated a clear discrepancy in immune reactivity between healthy donors and RA patients against the naturally occurring autoantigen HC gp-39. IFN-γ production by PBMC cultures from the majority of healthy donors that were stimulated by recall antigens could be inhibited by costimulation by HC gp-39. In contrast, the majority of such cultures from RA patients displayed an increase in IFN-γ-producing cells. Moreover, in contrast to HC gp-39-activated cells from RA patients that also produced IFN-γ, the HC gp-39-directed immune response of healthy individuals was hallmarked by the production of IL-10. IL-10 production depended on CD3-expressing cells, and CD4+ T cell lines against HC gp-39 displayed regulatory capacities.

These findings clearly indicate that healthy individuals, rather than being nonresponsive, frequently react to HC gp-39 with the production of IL-10. Our data can best be explained by the presence of HC gp-39-directed T_{reg} cells in healthy subjects because these cells have the potential to effectively suppress various proliferative and inflammatory responses.

The observations that the immune response of a considerable number of RA patients displays a proinflammatory polarization toward HC gp-39 and is less capable of inhibiting other immune responses are intriguing. Although some caution is required in interpreting these results because the lack of detecting suppression does not necessarily indicate the absence of suppressive responses, these data suggest that the inflammatory processes accompanying RA development/progression favor the induction of HC gp-39-directed Th1 cells. To become activated, naïve T cells have to encounter their specific antigens presented by HLA molecules on DCs. Although this event alone causes naïve T cell division, it does not necessarily establish a productive immune response because full mobilization to effector cells crucially depends on proper maturation of DCs (25). It is plausible that the inflammatory environment observed in the joints of RA patients meets the requirements for proper maturation of local DCs, enabling the induction of Th1 cell responses. Moreover, it has been reported that DCs present in the synovial tissue are able to present HC gp-39-derived peptides in the context of HLA class II molecules (11, 12). Therefore, we hypothesize that the emergence of IFN-γ-producing HC gp-39 directed T cells in RA patients is the result of HC gp-39 presentation

by maturated DCs that have the capacity to activate Th1 cells as a consequence of the maturation signals present in the inflamed synovium. In this way, the HC gp-39-specific Th1 cells are a result, rather than a cause, of the disease and could also be present in other (rheumatic) diseases.

The observation that a disease-associated bias is present with respect to the class of HC gp-39-directed immune response might point toward a more generalized phenomenon because it has recently been shown also that in diabetes and multiple sclerosis the autoreactive T cell response is polarized toward a proinflammatory Th1 phenotype, whereas a regulatory response is observed in health (26, 27). Although these observations have been made in different diseases, together these findings clearly indicate that induction of autoimmunity not only requires the emergence of pathogenic T cells (Th1 and/or Th2 cells) but also is associated with the loss of T_{reg} cell function that normally protects against autoimmunity. These findings are highly relevant for the development of antigen-specific intervention protocols because they suggest an approach for controlling immune responses. Particularly, RA might represent an autoimmune disease that is especially suited for targeting T_{reg} cells because, due to its systemic nature, a systemic approach is required to dampen the inflammatory reaction. It is likely that T_{reg} cells will focus on areas of inflammation, such as the inflamed joint, because they carry several receptors for inflammatory chemokines (5, 28–30). Indeed, the presence of T_{reg} cells in the inflamed joint, in addition to activated potentially pathogenic T cells, was recently demonstrated in synovial fluid of RA patients (31). Moreover, we have shown that CD4+ CD25+ T_{reg} cells are also involved in collagen-induced arthritis because depletion of CD4+ CD25+ T cells significantly increased severity and incidence of the disease, whereas reconstitution studies showed that infusion of CD4+ CD25+ T_{reg} cells conferred disease protection (32). The challenge for the future will be to induce and/or expand residual antigen-specific T_{reg} cell activity to treat autoimmune diseases in an antigen-specific manner.

Acknowledgements

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CHAPTER 10

Summary and discussion

Summary and discussion

Results in short

In this thesis several studies are described that include both clinical- and biological aspects in the field of undifferentiated **(part I)** and rheumatoid arthritis **(part II)**. **Chapter 2** describes the incidence and progression of undifferentiated arthritis to reumatoid artritis as monitored in different early arthritis clinics. Depending on the study population 6-55% of the patients who present with undifferentiated arthritis actually fulfill the criteria for rheumatoid arthritis as defined by the ACR in 1987 over time. For the prognosis of the first 4 years, it does not make a difference whether patients present with the clinical syndrome of an evident rheumatoid arthritis or with undifferentiated arthritis progressing to rheumatoid arthritis within one year **(chapter 3)**. Radiographic joint damage, disease activity and HAQ had a comparable course as measured over four years. The difference was not explicable by the duration of symptoms and thus time of the clinical phase, as median symptom duration was the same for both groups. Unfortunately, part of the window of opportunity might have been missed in the group of patients that presented with undifferentiated arthritis. In the PROMPT study, patients with undifferentiated arthritis were treated with either methotrexate or placebo. In the MTX group, the progression to rheumatoid arthritis was postponed, and radiographic damage was retarded. In patients with ACPAs the results were even more pronounced **(chapter 4 and 7)**. In PROMPT patients who had low and intermediate pretreatment ACPA-levels and were treated with MTX, the incidence of rheumatoid arthritis was lower than in patients with high levels. In a total group of DMARD-naive patients with undifferentiated arthritis or recently diagnosed rheumatoid arthritis, low and intermediate pretreatment ACPA-levels were associated with a more favorable response to MTX compared to patients with high levels of ACPA **(chapter 7)**. The treatment in patients with undifferentiated arthritis in PROMPT was aimed at a DAS <2.4, a scoring system that had been developed for patients with rheumatoid arthritis. Further analysis revealed that the use of DAS is justified in patients with undifferentiated arthritis **(Chapter 5)**. To identify which patient with undifferentiated arthritis has actually or will progress to rheumatoid arthritis and will thus benefit from early DMARD treatment, a prediction rule was developed. Based on sex, age, localization of symptoms, tender- and swollen joint count, C-reactive protein level, the severity of morning stiffness, and the presence of rheumatoid factor and ACPA, a prediction score with a cutoff level between 6 and 8 revealed a positive predictive value of 84% and a negative predictive value of 91% **(chapter 6)**. Treatment with DMARDs and biologicals are used to modulate immune responses in RA. Among other effects, it was shown that a TNF-alpha antagonist depleted activated CD4+CD25+ T cells by binding to membrane-bound TNF-alpha, resulting in recovery of the CD4+CD25+ regulatory T cells in patients with rheumatoid arthritis **(chapter 8)**. The

importance of the presence of functional regulatory T cells was emphasized in **chapter 9**. Whereas patients with rheumatoid arthritis reacted with a pro-inflammatory response to the human cartilage glycoprotein 39, healthy individuals had an anti-inflammatory response. The strength of the anti-inflammatory response was to such a degree that it even suppressed other pro-inflammatory responses.

Arthritis

It is unknown whether every patient with undifferentiated arthritis has the potential to develop rheumatoid arthritis. In this thesis, the assumption was made that some patients with undifferentiated arthritis actually had rheumatoid arthritis, which was not recognized as such yet. However, the clinical syndrome rheumatoid arthritis that fulfills the ACR criteria for rheumatoid arthritis can be seen as an end stage rheumatoid arthritis. Although the end product is destruction of the joint, the etiology may differ in this heterogeneous group. This heterogeneity is emphasized by the different onset of disease **(chapter 3)**.

Recently it has become clear that patients with ACPAs represent a distinct disease subset within the clinical syndrome rheumatoid arthritis (1;2). Having a shared epitope in the HLA class II molecules predisposes to the development of ACPAs (3;4). Most patients with ACPAs eventually show clinical characteristics of rheumatoid arthritis. However, it takes up to 13 years to progress to the clinical syndrome of rheumatoid arthritis (5;6). Somehow it requires an unidentified second hit to progress to clinical symptoms.

For patients without the presence of ACPA who have developed rheumatoid arthritis it is unclear which factors play a role. So far, no obvious genetic factor has been found. Like the danger model (7), one could propose that an undifferentiated arthritis for any reason could predispose to persisting arthritis if the danger signal persists, or even after a second hit may progress to a destructive rheumatoid arthritis. In that case, all patients with undifferentiated arthritis have the potential to progress to a higher level, rheumatoid arthritis, when the second event is provided. This is in contrast to the idea that undifferentiated arthritis at presentation is actually already rheumatoid arthritis. Because the biomarkers that make the difference have not been identified yet and the percentage of patients with undifferentiated arthritis that progresses to rheumatoid arthritis depends on the inclusion criteria of the study population, the prediction rule **(chapter 6)** is based on clinical symptoms and laboratory findings.

Monitoring undifferentiated arthritis

There are different ways to monitor rheumatoid arthritis, but most of them have not been validated in patients with undifferentiated arthritis. With the initiation of DMARDs early in the disease process, the outcome variables may be less pronounced, possibly requiring different methods for monitoring. Whereas in rheumatoid arthritis with a high

disease activity, the difference in improvement criteria and remission criteria is clear, in undifferentiated arthritis the improvement criteria partially resemble the remission criteria. Therefore, treatment goals should not be improvement, but remission in the most strict way.

In **chapter 5** it was shown that the use of the original DAS, which was used in the PROMPT study, was valid in patients with undifferentiated arthritis. The DAS28, however, may lead to an underestimation of disease activity if only few joints are involved that are not among the 28 counted, such as the feet. In undifferentiated arthritis, disease activity is generally low, and by using extended joint counts including the feet an underestimation of the number of joints involved may be prevented.

Although the DAS mirrors the disease activity in undifferentiated arthritis adequately, the translation to clinical practice should be defined. A DAS of more than 2.4 in patients with rheumatoid arthritis means an active disease state for most rheumatologists that is high enough to intensify medication (8). A DAS of 1.6 is used as a cut off for remission (9). However, accepting that a patient with initially undifferentiated arthritis who has a DAS less than 1.6 with still arthritis in at least 1 joint would be considered to be in remission is contradictory, as 1 swollen joint was enough to enter the PROMPT study. In 1981 the ARA remission criteria for rheumatoid arthritis were published. For remission, five or more of the following criteria must be fulfilled for at least two consecutive months: duration of morning stiffness not exceeding 15 minutes, no fatigue, no joint pain (by history), no joint tenderness or pain on motion, no soft tissue swelling in joints or tendon sheets, ESR less than 20 mm/h (male) or less than 30 mm/h (female) (10). Only 40% of the PROMPT patients with a DAS less than 1.6 fulfilled the ARA remission criteria. This is in contrast to patients with rheumatoid arthritis, where the DAS less than 1.6 correlates well with the ARA criteria for remission (9). This emphasizes that the definitions used for rheumatoid arthritis are not equally useful in undifferentiated arthritis. In the PROMPT study, remission was therefore defined as no clinical symptoms of arthritis and furthermore no DMARD use. The role of imaging in the definition of remission is up for debate. As ultrasound reveals more subclinical arthritis (11;12), one could argue that for remission and the treatment decision to stop DMARD therapy, even this subclinical arthritis should be absent. The predictive value on the long term is unclear and the advantages of continuing medication should be very carefully outweighed by the side-effects.

As erosive disease is very disabling, retarding bone erosions as quantified by radiographic damage is a very important outcome measurement. Studies in clinical rheumatoid arthritis show that the best effect of DMARDs on radiographic progression is when they are given in the first two years after rheumatoid arthritis is diagnosed (13-15). However, the majority of the patients in the PROMPT study had no radiographic joint progression in 18 months. If DMARD treatment is initiated in the window of opportunity before damage occurs, radiographic damage cannot be accurately measured. The lack

of radiographic damage alone is inconclusive. Repair of erosions was not scored in the PROMPT study, as it requires more patients with radiographic damage. Furthermore, it was reported that repair of erosions was observed in patients with absence of clinical synovitis for a longer period than the study time (16;17). As the radiographic damage was clearly seen in ACPA positive patients in the PROMPT study, radiographic monitoring should not be omitted, but for monitoring in early undifferentiated arthritis it is not the best measurement.

Treatment strategies in the window of opportunity

Once clinical symptoms of arthritis do occur, starting a treatment is justified. For patients who present with rheumatoid arthritis, DMARDs can be started immediately without discussion. However, in patients with undifferentiated arthritis, from which 6-55% actually has rheumatoid arthritis **(chapter 2)**, and thus 45-96% does not have rheumatoid arthritis, it is very important to withhold a potential toxic treatment with DMARDs for those who do not need it. To narrow down this very wide range a prediction rule was designed **(chapter 6)**. The ACR criteria for rheumatoid arthritis are not suitable for this purpose, as these criteria were designed for describing the population of patients with an established rheumatoid arthritis. Nor are the criteria for probable rheumatoid arthritis, as all the patients in the PROMPT study fulfilled the criteria for probable rheumatoid arthritis, and only 53% of the patients in the placebo group had reumatoid arthritis after 18 months. In 2002 a prediction model to discriminate between self-limiting, persistent non-erosive and persistent erosive arthritis was developed (18). In the present prediction rule, the aim was not to predict persistent arthritis, but to predict rheumatoid arthritis and the consequent justification for DMARD treatment. Depending on the cohort, no adequate prediction could be made in 6 to 25% of the patients. These results have been replicated in other cohorts (19-22). Although the prediction rule is a useful tool, there is still some space for improvement by adding other factors, like genetic or environmental, to simplify treatment decisions in the grey area in which no adequate prediction can be made.

In an ideal situation, DMARD treatment will induce remission before permanent damage has occurred. In the PROMPT study, the disease process could not be reversed, although the intervention was done in the presumed window of opportunity **(chapter 4)**. Maybe the duration of symptoms might have been too long for MTX to reverse the process or MTX monotherapy is just not enough. It is tempting to extrapolate the data from patients with early diagnosed rheumatoid arthritis to patients with undifferentiated arthritis who actually have rheumatoid arthritis. The BeSt- en COBRA-study showed that combination therapy is superior to monotherapy or step-up therapy (23;24). However, there is little evidence-based information on the choice of treatment of undifferentiated arthritis. Intra-articular corticosteroids in patients with oligoarthritis followed by

sulphasalazine in case of persistent or progressive disease resulted in more absence of synovitis at 1 year when compared with NSAIDs followed by the same delayed intervention if necessary (25). In patients with poor prognosis undifferentiated arthritis, which is recurrence of synovitis after a single dose of corticosteroids with symptom duration of less than 1 year, a randomized, placebo-controlled trial with infliximab provided modest short-term relief, but did not prevent the development of rheumatoid arthritis (26). Other double-blind randomized placebo-controlled studies in undifferentiated arthritis comprise treatment with antibiotics in mixed populations of undifferentiated arthritis and reactive arthritis and have had so far no effect on the disease course (27;28). Other open label studies with DMARDs in patients with undifferentiated arthritis were performed, but in mixed populations without a matched control group, and DMARDs were also initiated further in the disease process (29;30). So far the best treatment strategy for undifferentiated arthritis has not been found, though early intervention with DMARDs shows beneficial effects with regard to symptom reduction and postponing the clinical syndrome of rheumatoid arthritis.

ACPA-positive disease and ACPA-negative disease differ in histology, etiology and genetic background (1;2). Although the number of patients in the PROMPT study who were ACPA positive was small and it was a post-hoc analysis, the difference in the efficacy of methotrexate was striking. Moreover, in ACPA-positive patients, patients with low and intermediate levels of ACPA showed a more favorable response to MTX compared to patients with high levels of ACPA **(chapter 7)**. If this corresponds to an earlier phase in the development to the clinical syndrome rheumatoid arthritis, than a different treatment strategy for patients with low and intermediate levels might be more appropriate. There are no results of clinical trials with treatment strategies that distinguish between ACPA-positive and -negative disease. This will provide an extra dimension towards tailor-made treatment.

Immunomodulation

In autoimmune diseases the challenge is to stop the effect of the autoimmune processes. To achieve this goal most treatment strategies aim at suppressing the immune system by using agents that interfere with general processes like DNA synthesis, cytokinesignalling or receptorsignalling. Irrespective of the origin of the autoimmune disease or the affected organ these agents are successfully used. The counterpart of these agents is the incidence of infections due to the lack in a pro-inflammatory immune system. Biologicals, like anti-TNF-alpha, have had a major impact on the outcome of rheumatoid arthritis. It was shown that the suppressive capability of CD4+ CD25+ T cells was compromised in patients with active rheumatoid arthritis and was reversed after anti-TNF-alpha therapy (31;32). The CD4+CD25+ membrane-bound TNF-alpha-negative T cells showed suppressive capacities. Binding of adalimumab to membrane-bound

TNF-alpha resulted in depletion of activated CD4+CD25+ T cells and restoration of the suppressive capacity of the remaining functional CD4+CD25+ membrane-bound TNF-alpha-negative T cells. The presence of membrane-bound TNF-alpha on CD4+CD25+ T cells was correlated to disease activity in rheumatoid arthritis patients **(chapter 8).** This emphasizes the imbalance between the pro-inflammatory response and the regulatory T cell response in patients with rheumatoid arthritis. This imbalance was also found in the immunologic response to HC-gp39. T cells from rheumatoid arthritis patients produced anti-inflammatory cytokines like IFN-gamma in response to stimulation with HC-gp39, whereas T cells from healthy individuals reacted with producing IL-10. T cell lines against HC-gp39 from healthy individuals were able to suppress a pro-inflammatory response from a T cell line against a peptide from mycobacterium, suggesting that the response with IL-10 production was a regulatory response. Furthermore, the T cell lines against HC-gp39 from healthy individuals were CD4+ and expressed high levels of CD25 **(chapter 9)**.

Understanding the mechanisms in autoimmunity will provide tools in developing more specific treatment strategies. Autoimmune diseases occur in up to 3-5% of the general population. Both genetic and environmental factors contribute to the susceptibility to autoimmunity (33). In this thesis, T cell mediated immunoregulation is addressed. It is postulated that the immune system distinguishes between the absence and presence of danger signals in combination with either self or non-self peptides. In the presence of a danger signal, like pathogen-associated molecular patterns (PAMPs) binding to pattern recognition receptors and non-programmed cell death, the immune response is directed towards a pro-inflammatory response. In combination with a self peptide this will result in autoimmunity (7;34). The described imbalance between pro-inflammatory and regulatory immune responses implies that skewing the immune response from pro-inflammatory to anti-inflammatory or restoring the suppressor function at specific sites might invert the autoimmune process. As DMARDs and biologicals are broad spectrum agents that potentially react on every inflammation in the body, the ultimate challenge in autoimmunity will be to interfere within the immune processes more specifically. HC-gp39 might be a candidate auto antigen to target for redirecting the immune response as it is one of the major constituents of human cartilage, the mRNA and protein are upregulated in synovium of rheumatoid arthritis patients and T cell-epitopes from HC gp-39 are presented by antigen presenting cells in synovium of rheumatoid arthritis patients (35-39). In a mouse model, intranasal treatment with HC-gp39 suppressed HC-gp39-induced and collagen-induced arthritis (40;41). However, tolerance induction in rodents is possible via specialised superficial cervical and internal jugular draining lymphnodes of the nose. Other lymfnodes do not have this capacity (42). It is difficult to perform such controlled experiments with respect to dosing and stage of the disease in

humans. Here, it was shown that the response to HC-gp39 differs between healty donors and rheumatoid arthritis patients.

In an attempt to influence the immunological response to HC gp-39 in order to influence disease outcome, a phase I trial was performed. Briefly, rheumatoid arthritis patients with moderate disease activity were treated with once weekly 25 µg, 125 µg, 625 µg or 3125 µg HC gp-39 intranasally during 4 weeks, and followed for another 8 weeks. In every treatment group, eight patients received HC gp-39 and two patients received placebo. No severe adverse events were reported, however, a clinical significant effect on the disease activity of the rheumatoid arthritis patients was not seen either (43). To determine the underlying immunological reaction, peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved from patients with RA who participated in the phase I clinical trial. Blood samples were taken before entering the trial, after approximately 4 weeks of treatment and after an 8-week HC gp-39 free interval being approximately 12 weeks after entering the trial. The HC gp-39 response was not tested before entering the trial. PBMCs of only 23 patients were available for analysis. Difference at the level of cytokine production as measured by cytometric bead arrays and in enzyme linked immuno sorbent culture did not distinguish the placebo group from the four treatment groups (H. van Dongen, unpublished results). The obtained results were not elegible for publication for several reasons. Blood samples from only 23 out of 40 patients were available for the analysis. As the remaining initial groups were too small for correct statistical testing, a proof of principle would be the only possibility. After selecting the patients based on clinical outcome, the groups were also too small, and the results differed per patient. Induction to tolerance against HC-gp39 in humans has not been performed yet. The fact that HC-gp39 is probably not the antigen that drives the expansion of T cells to an autoimmune process should be irrelevant as long as the response to HC-gp39 is strong enough to suppress the other immune responses in the joint. This might even be an advantage, as a reduced frequency of nickel hypersensitivity was reported after oral nickel contact at an early age by oral braces. The reduced frequency was only seen if the oral contact was prior to sensitization to nickel by i.e. earpearcing (44). Perhaps HC-gp39 should be administered to patients in rheumatoid arthritis who do not show a pro-inflammatory response to HC-gp39 (yet), to reinforce the existing anti-inflammatory response and will hopefully suppress other inflammatory responses as HC-gp39 is up regulated in the inflamed synovium. In mice, collagen type II induced arthritis is also best prevented when the feeding of collagen type II starts before the induction of arthritis. Studies concerning oral tolerance induction in rheumatoid arthritis patients have been performed, but clinical efficacy was poor and functional evidence of tolerance was not convincing, as cytokine profiles and markers like FoxP3 and CD25 are not specific enough, or even absent (45;46). Therefore, no conclusions could be drawn.

Future perspectives

Undifferentiated arthritis remains a challenging state of disease. The outcome varies from self limiting to severe destructive arthritis. As the evidence for early treatment of rheumatoid arthritis is accumulating, the urge for selecting patients early in the disease course increases. As genetic susceptibility is already present at birth and ACPA can be detected up to 14 years before clinical symptoms of arthritis occur, there will be no support for starting a treatment in the preclinical phase (5;6). The choice of treatment should be crystallized in clinical trials in patients with undifferentiated arthritis. When patients with rheumatoid arthritis are distinguishable in a population of patients with undifferentiated arthritis, and the immunologic reaction is not widespread, perhaps oral tolerance induction will be possible. Many candidate auto antigens have been investigated, but most of them do not make it from bench to bedside. For HC-gp39 the curtains haven't closed yet. More translational research regarding this aspect is necessary.

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CHAPTER 11

Nederlandse samenvatting

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Het onderzoek dat beschreven wordt in dit proefschrift bestaat uit twee delen: **Deel I** gaat over ongedifferentiëerde artritis, de progressie daarvan naar reumatoïde artritis, het resultaat van agressieve behandeling in een vroeg stadium van reumatoïde artritis en hoe te voorspellen welke patiënten daarvoor in aanmerking komen. In **deel II** worden enige immunologische aspecten van reumatoïde artritis beschreven.

Deel I: Ongedifferentiëerde artritis

Artritis

Een gewrichtsontsteking of artritis wordt gekenmerkt door warmte, pijn en zwelling in een of meerdere gewrichten. Reumatoïde artritis is de meest voorkomende chronisch inflammatoire gewrichtsaandoening en komt bij 1% van de bevolking voor, waarbij meer vrouwen dan mannen zijn aangedaan. Het is belangrijk om reumatoïde artritis in een vroeg stadium te herkennen, omdat deze ziekte onbehandeld uiteindelijk leidt tot destructie van het gewricht. Door het starten van behandeling zodra de diagnose reumatoïde artritis duidelijk is, kan deze schade voorkomen worden. Om reumatoïde artritis in een vroeg stadium te onderzoeken, zijn zogenaamde 'early artritis clinics' opgezet. Vanaf de jaren 50 zijn er criteria ontworpen om de verschillende vormen van artritis in te delen. Hierbij worden o.a. het voorkomen van ochtendstijfheid, het soort en aantal aangedane gewrichten, de aanwezigheid van antilichamen zoals reumafactor, en al bestaande gewrichtsschade beoordeeld (zie ook tabel 1, **hoofdstuk 1**). Als een artritis niet valt te categorizeren in een van de ziekteëntiteiten die is gedefinieerd door de American College of Rheumatology (ACR), betreft het een ongedifferentiëerde artritis. Uit de Leidse 'early artritis clinic' bleek dat 37% van de patiënten die de polikliniek bezocht met een artritis een ongedifferentiëerde artritis had. Na een jaar had 30% van deze patiënten geen artritis meer. 28% bleek echter een reumatoïde artritis te hebben. In **hoofdstuk 2** is onderzocht hoe vaak patiënten met ongedifferentiëerde artritis in verschillende 'early artritis clinics' wereldwijd binnen 1 jaar reumatoïde artritis ontwikkelden. Afhankelijk van het soort en het aantal gewrichten dat is aangedaan bleek 6-55% van de patiënten met een ongedifferentiëerde artritis binnen 1 jaar reumatoïde artritis te hebben. Indien werd vereist dat patiënten bij een eerste beoordeling door een reumatoloog een artritis hadden en dat voor de diagnose reumatoïde artritis moest worden voldaan aan de classificatie criteria zoals opgesteld door de ACR, bleek 17-32% van de patiënten binnen 1 jaar reumatoïde artritis te hebben.

Beoordelen en vervolgen van artritis

Reumatoïde artritis wordt gekenmerkt door toenemende destructie van gewrichten, die uiteindelijk tot invaliditeit leiden. Er zijn verschillende methoden om de ernst van een artritis en de ziekte activiteit te beoordelen en te vervolgen. Deze methoden kunnen gebruikt worden om de effectiviteit van therapie te beoordelen, of als uitgangspunt om wel of geen medicatie te starten.

De 'disease activity score', kortweg DAS, is een formule die is gebaseerd op een aantal pijnlijke gewrichten, een aantal gezwollen gewrichten, de hoogte van een ontstekingsparameter in het bloed (bezinking), en de beoordeling van algemeen welbevinden op een schaal van een tot tien door de patiënt. De DAS was gebaseerd op ziekteactiviteit in patiënten met reumatoïde artritis. In **hoofdstuk 5** blijkt dat de DAS score ook toepasbaar is in patiënten met een ongedifferentiëerde artritis. In patiënten met reumatoïde artritis is een DAS van 2,4 reden om medicatie op te hogen, en een DAS van 1,6 wordt gebruikt als afkapwaarde voor remissie. Echter in patiënten met een ongedifferentiëerde artritis, die per definitie minder pijnlijke en gezwollen gewrichten hebben, kan de DAS 1,6 zijn terwijl er artritis is. Met de huidige behandelstrategieën en het toenemend bewijs voor het starten van agressieve therapieën vroeg in het ziekte proces zal de definitie van remissie, al dan niet op basis van de DAS, voor patiënten met ongedifferentiëerde artritis aangepast moeten worden.

Beeldvorming van het aangedane gewricht speelt ook een rol in de beoordeling van de ernst van de reumatoïde artritis. De Sharp-van der Heijde gemodificeerde scoringsmethode is een manier om röntgenfoto's van handen en voeten te beoordelen op het optreden van gewrichtsspleetversmalling en erosies (zie figuur 1, **hoofdstuk 1**). Hoe hoger de score, des te meer schade aan het gewricht. Deze manier van beoordelen is ook gebruikt in de onderzoeken beschreven in dit proefschrift.

Andere manieren om de ernst van de ziekte te beoordelen zijn meer subjectieve methoden met vragenlijsten naar algemeen functioneren, zoals de 'health assessment questionnaire' (HAQ), de 'arthritis impact measurement scales' (AIMS), en de 'rheumatoid arthritis disease activity index' (RADAI).

Beargumentatie voor behandeling vroeg in het ziekteproces

De afgelopen twintig jaar is de behandeling van reumatoïde artritis sterk veranderd. Voor 1994 werd een patiënt met nieuw gediagnostiseerde reumatoïde artritis behandeld volgens de pyramide strategie. Er werd dan begonnen met een ontstekingsremmer, een zogenaamde 'non-steroid anti-inflammatory drug' (NSAID) en pas bij ernstige ziekte werd gestart met een 'disease-modifying anti-rheumatic drug' (DMARD). Met een NSAID werden wel de klachten verminderd, maar van NSAIDs wordt niet gedacht dat ze het onderliggende ziekteproces beïnvloeden zoals bij DMARDs. Later bleek dat patiënten die bij de diagnose reumatoïde artritis gelijk startten met een DMARD minder schade

aan de gewrichten op rontgenfoto's van handen en voeten en een lagere ziekteaktiviteit hadden dan patiënten behandeld volgens de pyramide strategie. Het grootste effect werd daarbij in het eerste jaar van behandeling gezien. Na 10 jaar was dit effect nog steeds zichtbaar in het aantal operaties dat nodig was als gevolg van opgelopen gewrichtsschade. Het lijkt er dus op dat hoe eerder er in het ziekteproces ingegrepen wordt, des te beter de uitkomst op lange termijn is. In **hoofdstuk 3** is onderzocht of patiënten die zich presenteren met een ongedifferentiëerde artritis en binnen een jaar reumatoïde artritis ontwikkelen een milder ziektebeeld hebben dan patiënten die zich presenteren met een duidelijke reumatoïde artritis. Het blijkt dat in een tijdsbestek van vier jaar de patiënten die in het begin een ongedifferentiëerde artritis hadden net zo veel gewrichstbeschadiging en ziekteactiviteit hadden ontwikkeld als diegenen die in het begin een duidelijke reumatoïde artritis hadden. Wanneer het grootste effect van behandeling in het eerste jaar wordt gezien, zou het starten van DMARDs al in het stadium van ongedifferentiëerde artritis gerechtvaardigd zijn. Deze aanname is onderzocht in de *PRO*bable RA: *M*ethotrexate versus *P*lacebo *T*reatment (PROMPT) studie (**hoofdstuk 4**). In de PROMPT studie zijn patiënten met een ongedifferentiëerde artritis behandeld met methotrexaat of placebo. In de methotrexaat groep bleek dat progressie van het ziektebeeld naar een duidelijke reumatoïde artritis uitgesteld was en dat er minder schade op röntgenfoto's te zien was dan in de placebo groep. In patiënten met antistoffen tegen gecitrullineerde peptiden (ACPAs) waren de resultaten nog meer uitgesproken **(hoofdstuk 4 and 7)**.

Recent is duidelijk geworden dat patiënten die ACPAs hebben een aparte groep vormen binnen de groep patiënten met een reumatoïde artritis. Het hebben van een bepaalde gezamelijke aminozuursequentie, de zogenaamde 'shared epitope', in human leucocyte antigen (HLA) klasse II moleculen is een aanleg voor het ontwikkelen van ACPAs. De meeste patiënten met ACPAs ontwikkelen uiteindelijk reumatoïde artritis. Het kan echter wel 13 jaar duren voor dit plaats vindt. Op een of andere manier is er een onbekende tweede factor nodig om klinische verschijnselen te ontwikkelen (zie figuur 2, **hoofdstuk 1**). Voor patiënten die geen ACPAs hebben, maar wel reumatoïde artritis, is het nog onduidelijk welke andere factoren een rol spelen.

Binnen de PROMPT studie was de incidentie van reumatoïde artritis lager in patiënten die lage of intermediaire ACPA titers hadden en behandeld werden met methotrexaat dan in patiënten met een hoge antistoftiter. In een groep patiënten die nooit eerder was behandeld met een 'disease-modifying anti-rheumatic drug' (DMARD) en gediagnostiseerd waren met ongedifferentiëerde artritis of een net ontstane reumatoïde artritis bleek dat lage en intermediaire ACPA titers geassocieerd waren met een betere reactie op methotrexaat behandeling dan patiënten met hoge antistoftiters **(hoofdstuk 7)**.

Om te bepalen welke patiënt met een ongedifferentiëerde artritis eigenlijk reumatoide artritis heeft, of waarschijnlijjk zal ontwikkelen, en dus wie baat zou hebben bij een

vroege behandeling met een DMARD, is een voorspelregel ontwikkeld. Op basis van geslacht, leeftijd, plaats van de klachten, het aantal pijnlijke en gezwollen gewrichten, de hoogte van het C-reactieve proteine, de mate van ochtendstijfheid, en de aanwezigheid van reumafactor en ACPA leverde een voorspellende score met afkapwaarden tussen de 6 en 8 punten een positief voorspellende waarde van 84% en een negatief voorspellende waarde van 91% op **(hoofdstuk 6)**.

Deel II: Reumatoïde artritis

Reumatoïde artritis is een zogenaamde auto-immuunziekte waarbij het immuunsysteem zorgt voor een ontstekingsproces in de gewrichten met als eind resultaat destructie van het gewricht. De gedachte is dat door een combinatie van genetische factoren en uitlokkende omgevingsfactoren schadelijke T cellen worden geactiveerd die onder andere B cellen kunnen stimuleren en daarmee de antistofproductie op gang brengen (zie figuur 2, **hoofdstuk 1**). Verschillende onderdelen van deze cascade van gebeurtenissen zijn onderzocht.

De invloed van genetisch factoren blijkt uit een drie tot vier keer zo vaak voorkomen van reumatoïde artritis in een-eïge in vergelijking tot twee-eïge tweelingen. De totale genetische bijdrage wordt geschat op 50 tot 60 %. De meest bekende risicofactor tot nu toe is de 'shared epitope' in het HLA klasse II gen.

T cellen

De betrokkenheid van HLA klasse II moleculen en de aanwezigheid van T cellen gericht tegen eiwitten afkomstig uit het gewricht suggereren een rol voor CD4+ T cellen. Nadat de T cellen zijn onstaan uit voorlopercellen in het beenmerg, verhuizen ze naar de thymus om daar het onderscheid te leren maken tussen lichaamsvreemde en lichaamseigen eiwitten, gepresenteerd in eigen HLA moleculen. Allereerst vindt positieve selectie plaats van die T cellen die peptiden herkennen in de context van eigen HLA moleculen. Daarna vindt een negatieve selectie procedure plaats waarbij T cellen die reageren op lichaamseigen eiwitten geëlimineerd worden. Grofweg bestaan er twee typen T cellen. Cytotoxische T cellen die gekenmerkt worden door CD8 expressie en die antigenen herkennen in de context van HLA klasse I moleculen. Deze moleculen zitten op vrijwel alle cellen met een celkern en presenteren intracellulaire eiwitten. Als de CD8+ T cel geaktiveerd wordt, doodt de T cel de antigeen presenterende cel door cellysis. Tijdens aktivatie van CD8+ T cellen komen pro-inflammatoire cytokinen als interferon-gamma, tumor necrosis factor- alfa en interleukine-2 vrij. T helper cellen brengen CD4 op hun celmembraan tot expressie en herkennen antigenen in de context van HLA klasse II. Alleen antigeen presenterende cellen als dendritische cellen, monocyten, macrofagen

en B cellen kunnen extracellulaire eiwitten opnemen, bewerken en presenteren in HLA klasse II moleculen. Wanneeer de T helper cel geactiveerd wordt, kan deze B cellen activeren die vervolgens van bepaalde antistoffen gaan produceren. Deze processen worden gekenmerkt door het vrij komen van interleukine-4 en interleukine-10.

Bovenstaande processen verklaren echter niet waarom regulatoire of suppressor T cellen bestaan en waarom er autoimmuunziekten kunnen ontstaan. Er bestaan verschillende hypothesen over de origine van autoimmuniteit, variërend van genetische defecten die het selectie proces in de thymus beinvloeden tot 'danger models' waarin beschadigde weefsels een waarschuwingssignaal afgeven, waarbij dit waarschuwingssignaal fungeert als co-stimulerende factor voor activatie van een autoimmuunproces. Het bestaan van regulatoire T cellen is functioneel voor het eerst aangetoond in naakte muizen waarbij infusie van CD25+CD4+ T cellen na infusie van CD25-CD4+ T cellen voorkwam dat er een autoimmuunziekte ontstond. CD25 is de alfa keten van de interleukine-2 receptor. Bij analyse met behulp van flowcytometrie komt CD25 hoog tot expressie op geactiveerde T cellen vroeg in de activatiefase, terwijl er een intermediair expressie patroon op regulatoire T cellen wordt beschreven. Ander celmarkers, zoals 'cytotoxic T lymphocyte antigen-4' (CTLA-4), 'glucocorticoid induced TNF receptor (GITR) en FoxP3 worden ook wel gebruikt om regulatoire T cellen te beschrijven, maar deze markers zijn ook niet specifiek. De regulatoire T cel reactie wordt gekenmerkt door het vrijkomen van anti-inflammatoire cytokinen, zoals interleukine-10 en tumor groei factor-beta. T cel gemedieerde immunoregulatie speelt waarschijnlijk wel een rol in de tolerantie van lichaamseigen eiwitten, maar de exacte rol van regulatoire T cellen blijft onduidelijk, vooral in de mens.

De uitdaging in autoimmuunziekten is het beëindigen van het autoimmuunproces. Behandeling met DMARDs en zogenaamde 'biologicals', zoals TNF-alfa antagonisten, worden gebruikt om de immunologische processen in onder andere patiënten met reumatoïde artritis te beinvloeden. Het bleek dat de suppressieve capaciteit van CD4+CD25+ T cellen in patiënten met reumatoïde artritis was verminderd, en weer hersteld na anti-TNF-alfa therapie. In **hoofdstuk 8** bleek dat de aanwezigheid van membraangebonden TNF-alfa op CD4+ CD25+ cellen samenhing met ziekteactiviteit in patiënten met reuamatoide artritis. Binding van een gehumaniseerde TNF-alfa antagonist, adalimumab, aan membraangebonden TNF-alfa resulteerde in het uitschakelen van geactiveerde CD4+CD25+ T cellen, waardoor de functie van CD4+CD25+ regulatoire T cellen hersteld werd. Dit benadrukt de onevenwichtigheid tussen de pro-inflammatoire reaktie en de regulatoire T cel reactie in patiënten met reumatoïde artritis.

Deze onevenwichtigheid wordt ook gevonden in de immunologische reactie op het humane kraakbeen glycoproteïne-39 (HC gp-39). In **hoofdstuk 9** blijkt dat patiënten met reumatoïde artritis een pro-inflammatoire reactie hadden op HC gp-39, terwijl gezonde personen een anti-inflammatoire reactie hadden. De sterkte van deze anti-inflammatoire

reactie was zo groot dat deze andere pro-inflammatoire reacties kon onderdrukken. Bovendien maakt HC gp-39 een groot deel uit van het humane kraakbeen, het mRNA en het eiwit zelf komen verhoogd tot expressie in het synovium van patiënten met reumatoïde artritis en delen van HC gp-39 worden door antigeen presenterende cellen in het synovium van patiënten met reumatoïde artritis gepresenteerd aan T cellen. HC gp-39 zou daarmee een potentiëel middel kunnen zijn om het autoimmuunproces in reumatoïde artritis te beïnvloeden. In een muismodel bleek dat intranasaal toedienen van HC gp-39 het ontstaan van artritis kon onderdrukken. Naar analogie hiervan werd een fase I studie in patiënten met reumatoïde artritis opgezet, waarbij HC gp-39 gedurende 4 weken wekelijks werd toegediend. Daarna werden de patiënten nog 8 weken beoordeeld op ziekte activiteit. Helaas bleek er klinisch geen duidelijk effect waarneembaar. Om onderliggende immunologische reacties te analyseren, zijn tijdens deze fase I studie bloedmonsters afgenomen. In de **Summary and discussion** worden de resultaten van deze analyse besproken. Helaas was er voor deelname aan de fase I studie niet getest op de aanwezigheid van een pro-inflammatoire reactie op HC gp-39, waardoor de groep patiënten zeer heterogeen bleek. Daar niet van alle patiënten een monster beschikbaar was, en per type HC-gp-39 reactie te weinig patiënten per groep waren, was het niet mogelijk om hier een goede conclusie uit te trekken. Daarbij komt nog dat knaagdieren mogelijk een ander werkingsmechanisme van tolerantie inductie hebben dan mensen, er geen duidelijk klinisch effect waarneembaar was in de patiënten in de fase I studie, en het niet duidelijk is of in de fase I studie de best mogelijke doseringen zijn gebruikt. Derhalve zijn de resultaten van dit laatste project niet gepubliceerd. Dat tolerantie inductie mogelijk is bij mensen blijkt uit het feit dat bij mensen die op jonge leeftijd een beugel of piercing gehad hebben, op latere leeftijd minder vaak nikkel allergieën voorkomen.

Toekomstperspectieven

Ongedifferentiëerde artritis blijft klinisch een enorme uitdaging. Er is steeds meer bewijs dat vroeg starten van een behandeling in patiënten met reumatoïde artritis zeer gunstige effecten heeft op het latere beloop van de ziekte. Des te belangrijker is het om de reumatoïde artritis patiënt al in een vroeg stadium te herkennen. De keuze van therapie lijkt vooralsnog verschillend te zijn in verschillende stadia van de ziekte. Waar het beste mee gestart kan worden in de fase van een ongedifferentiëerde artritis zal nog verder uitgezocht moeten worden. Tevens lijkt de aanwezigheid van ACPAs al in een vroeg stadium van belang. Het is nog niet uitgesloten dat orale tolerantie inductie een mogelijkheid is, en ook voor HC gp-39 zouden er theoretisch nog mogelijkheden zijn.

Curriculum vitae

Henrike werd op 16 december 1975 geboren in Dordrecht. In 1994 werd het V.W.O.-diploma behaald aan Dordtwijck, locatie Noordendijk, te Dordrecht, waarna zij startte met de studie Biomedische Wetenschappen aan de Universiteit Leiden. In 1996 startte zij tevens met de studie Geneeskunde aan de Universiteit Leiden. Zij heeft een wetenschapsstage gelopen bij de afdeling Reumatologie (begeleiding: prof. dr. T.W.J. Huizinga, prof. dr. C.L. Verwey, dr. E.L. Kaijzel) getiteld 'Associative and functional aspects of four interleukin-1 gene polymorphisms in rheumatoid arthritis', resulterend in een publicatie. In Dormaa Presby Hospital, Dormaa Ahenkro, Ghana, heeft zij een klinische stage gelopen (begeleiding: drs. C. Brumsen, A. Owusu Ansah). Haar afstudeerstage vond plaats bij de Afdeling Immunologische en Infectieziekten, TNO Preventie en Gezondheid te Leiden, getiteld 'Alpha B-crystallin as key autoantigen in a mouse model for multiple sclerosis' en eveneens resulterend in een publicatie (begeleiding: dr. J.M. van Noort, dr. R. Verbeek). De doctoraalexamens Biomedische Wetenschappen en Geneeskunde werden in 2001 behaald, waarna het artsexamen in 2003 volgde.

In april 2003 ving zij aan met het in dit proefschrift beschreven promotieonderzoek bij de afdeling Reumatologie onder begeleiding van prof. dr. T.W.J. Huizinga en prof. dr. R.E.M. Toes, waarvoor zij een persoonsgebonden ZonMw AGIKO-stipendium ontving. Voor een deel van dit onderzoek werd zij genomineerd voor de Abott Young Investigator Award in 2005 en ontving zij een Eular Abbott Abstract Award in 2006. Vanaf 2003 volgt zij de postdoctorale opleiding tot immunoloog van de Stichting opleiding tot Medisch-Biologisch Wetenschappelijk Onderzoeker (SMBWO).

Van mei 2006 tot en met december 2008 is zij gestart als arts in opleiding tot specialist (AIOS) in de Reumatologie bij de afdeling Interne geneeskunde in het Medisch Centrum Haaglanden te Den Haag (opleider mw. dr. P.H.L.M. Geelhoed-Duijvestijn). Van januari 2009 tot en met april 2010 heeft zij haar vooropleiding Interne geneeskunde vervolgd in het Leids Universitair Medisch Centrum (LUMC) op de afdeling Interne Geneeskunde (opleider prof. dr. J.A. Romijn). Vanaf 1 mei 2010 is zij werkzaam als AIOS Reumatologie op de afdeling Reumatologie in het LUMC (opleider prof. dr. T.W.J. Huizinga).

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