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RISK FACTORS FOR RHEUMATOID ARTHRITIS: EPIDEMIOLOGICAL STUDIES OF PERINATAL CHARACTERISTICS, INFECTIONS AND THE INFLAMMATORY REFLEX

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Cover: Detail from Le Moulin de la Galette, 1876, by Pierre-August Renoir, Renoir suffered from rheumatoid arthritis the last thirty years of his life. Despite an aggressive disease leading to severe deformities and progressive disability he continued to paint and roll his cigarettes.

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterised by symmetric polyarthritis leading to progressive joint destruction. The aim of this thesis was to contribute to better knowledge of the etiology of RA by investigating the importance of the inflammatory reflex, childhood infections and early life exposures as risk factors for RA, based on four epidemiological studies.

In the first study we explored the importance of birth characteristics and infections during the first year of life on the risk of later RA and juvenile idiopathic arthritis (JIA). Using data from the Swedish Hospital Discharge Register, the Swedish Early Rheumatoid Arthritis Register and the Swedish Medical Birth Register, we conducted two parallel case-control studies on 333 cases of "early adult onset" RA and 3334 cases of JIA, and four matched controls per case. We noted an increased risk for JIA and rheumatoid factor (RF) negative RA following hospitalisation for infection during the first year of life. A somewhat reduced risk for RA was suggested for low birth weight, being small for gestational age and preterm birth.

In the second study we aimed to confirm, in a larger study population, the role of infections during early childhood on the risk of developing RA and to extend the assessment of infections to encompass infections throughout childhood. We performed a case-control study using 3038 RA cases, born 1949 or later, identified in the Early Rheumatoid Arthritis Register, and five matched population based controls per case. Exposure information was retrieved from the Swedish Hospital Discharge Register. Overall, hospitalisation for any infection before 16 years of age was associated with an increased risk of developing RA. No overall difference was noted between RF positive and RF negative RA although RF negative RA was associated with infections during the first year of life and RF positive RA was associated with infections at 8 to15 years.

In the third study we aimed at exploring the role of the inflammatory reflex on the risk of RA. In two parallel case-control studies of 63 092 prevalent and 2548 incident RA cases identified in the Swedish Hospital Discharge Register and Swedish Early Rheumatoid Arthritis Register respectively, and matched population based controls, we assessed the association between surgical vagotomy and subsequent risk of developing RA. Vagotomy had no effect on the risk of developing RA.

In the fourth study we searched to investigate the role of the inflammatory reflex in humans indirectly, through the effect of nicotine. Using a design where we compared disease risks associated with smoking and with use of moist snuff, we aimed to disentangle the role of nicotine from the effect of other inhaled components of tobacco smoke on the development of chronic inflammation. We conducted a retrospective cohort study on the risk of RA, ulcerative colitis, Crohn's disease, sarcoidosis and multiple sclerosis associated with smoking and use of moist snuff, using a uniquely large cohort of 277 777 Swedish construction workers, who provided prospectively collected information on tobacco using habits between 1978 and 1993. Cross-linkage to the Swedish Hospital Discharge Register provided information on occurrence of disease outcome. Our results confirm and extend previous observations that smoking is a risk factor for (RF positive) rheumatoid arthritis, Crohn's disease and multiple sclerosis, that quitting smoking reduces these risks, that smoking cessation increases the risk of ulcerative colitis and that smoking reduces the risk of sarcoidosis. In sharp contrast, we noted no evidence of any association between use of moist snuff and the

risk of either of these chronic inflammatory diseases, with the exception of a borderline increased risk for MS, together indicating no major effect of the inflammatory reflex in the etiology of chronic inflammatory diseases.

LIST OF PUBLICATIONS

I. **Carlens C,** Jacobsson L, Brandt L, Cnattingius S, Stephansson O, Askling J. Perinatal characteristics, early life infections and later risk of rheumatoid arthritis and juvenile idiopathic arthritis. Ann Rheum Dis 2009;68;1159-1164; originally published online 28 Oct 2008.

II. **Carlens C,** Holmqvist ME, Alfredsson L, Klareskog L, Askling J. Infections during childhood and later risk of rheumatoid arthritis. Manuscript

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IV. **Carlens C,** Hergens M-P, Grunewald J, Ekbom A, Eklund A, Olgart Höglund C, Askling J. Smoking, use of moist snuff and risk of chronic inflammatory diseases. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Antibodies to citrullinated protein antigens
ACR	American College of Rheumatology
AM	Alveolar macrophage
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
CCP	Cyclic citrullinated peptide
CD	Crohn's disease
CI	Confidence Interval
CTL	Cytotoxic T-cell
DAS	Disease activity score
DOHaD	Developmental Origins of Health and Disease
ERAR	Early Rheumatoid Arthritis Register
HLA	Human leukocyte antigen
ICD	International Classification of Diseases
IL	Interleukin
ILAR	International League of Association for Rheumatology
INF	Interferon
JIA	Juvenile idiopathic arthritis
MHC	Major histocompatibilty complex
MS	Multiple sclerosis
nAChR	Nicotinic Acetylcholine Receptor
NRN	National registration number
OR	Odds ratio
PAMP	Pathogen associated molecular pattern
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SD	Standard deviation
SE	Shared epitope
TCR	T-cell receptor
Th	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T regulatory cell
UC	Ulcerative colitis

1 INTRODUCTION

Rheumatoid arthritis occurs worldwide. Approximately 24 million people suffered from RA in 2004 [1]. In Sweden close to 60 000 people suffered from RA in 2008.

Despite intensive research during the last decades, the etiology of rheumatoid arthritis (RA) and other chronic inflammatory diseases remains an enigma. Supposedly, disease development is the result of an interplay between genetic susceptibility, environmental risk factors, and stochastic events. The aim of this thesis was to broaden our understanding of the etiology of RA.Although it is clear that environmental factors do affect the risk of developing RA, little is known about when they are of importance. Observations such as the presence of ACPA as well as RF several years before clinical onset of RA, suggest that critical risk factors may act much earlier than previously thought.

Numerous epidemiological and animal studies during the past decades have suggested that the environment during fetal and infant life is of importance for the risk of later development of several different types of diseases including autoimmune diseases such as coeliac disease and type I diabetes mellitus. The scarce data so far available in the context of RA do, however, strengthen the hypothesis that factors early in life may be of importance in the etiology of RA. Taking advantage of existing nation-wide population-based registers and a clinical register of early RA we performed two case-control studies investigating the importance of perinatal characteristics and childhood infections on the risk of later RA and juvenile idiopathic arthritis (JIA).

Blockade of tumour necrosis factor (TNF) has proven to be a highly successful treatment for RA. Numerous animal studies have shown the existence of an "inflammatory reflex", a physiological pathway acting via the vagus nerve, regulating immune response. The efferent part of the inflammatory reflex, termed the cholinergic anti-inflammatory pathway, mediates its effect through α7 nicotinic acetylcholine receptors, inhibiting the production of pro-inflammatory cytokines such as TNF, thus exerting an anti-inflammatory effect. Decreased activity in the cholinergic anti-inflammatory pathway might result in suppressed ability to counteract excessive cytokine expression, and thereby facilitate development of autoimmune reactions, which in genetic susceptible individuals may subsequently lead to development of chronic conditions such as RA and other chronic inflammatory diseases. We therefore performed two studies exploring the impact of the inflammatory reflex on the risk of developing RA and other chronic inflammatory diseases. First we assessed the risk of RA after surgical vagotomy in a case-control study. We then performed a large retrospective cohort study using a uniquely large cohort of about 300 000 contruction workers, on the risk of RA, ulcerative colitis (UC), Crohn's disease (CD), sarcoidosis and multiple sclerosis (MS) associated with smoking and use of moist snuff with the aim to evaluate the effect of nicotine and thus indirectly the importance of the inflammatory reflex.

2 DISEASES STUDIED IN THIS THESIS

2.1 RHEUMATOID ARTHRITIS

2.1.1 Clinical course and classification

Rheumatoid arthritis (RA) is a chronic inflammatory disease, characterised by symmetric polyarthritis leading to progressive joint destruction. About 70% of the patients with RA are women. The disease onset is often insidious, lasting a couple of months, presenting with a symmetric inflammation of the small joints of the hands and feet with persistent pain accompanied by pronounced morning stiffness, tiredness and weight loss. The clinical course is heterogeneous and difficult to predict, ranging from a mild, self-limiting disease to a rapidly progressive multisystem inflammation. If untreated, the majority of patients will experience disease progression including cartilage breakdown and bone erosions resulting in joint destruction and functional impairment. RA also has an impact on emotional and social functioning and often results in working disabilities and a reduction in quality of life already early in the disease course [2, 3]. In addition numerous studies have demonstrated a reduced life expectancy in patients with RA, mainly due to increased morbidity from cardiovascular disease, but also from infections and cancer [4-6].
There is no single test defining RA. Instead, RA is a diagnosis based on shared clinical, laboratory and radiological features. Most often the diagnosis is based on the American Collage of Rheumatology 1987 revised classification criteria, although these were not developed to be used in clinical practice [7], table 1. During the last decade, new treatments have shown an impressive ability to slow down disease progression, prevent joint destruction, and even induce remission, in particular if started early in the disease course. Because of this "window of opportunity" early diagnosis is crucial and new diagnostic tools are needed.

Table 1 A patient shall be said to have RA 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.

	ACR 1987 criteria
1	Morning stiffness
2	Arthritis of three or more joints
3	Arthritis of hand joints
4	Symmetric arthritis
5	Rheumatoid nodules
6	Serum rheumatoid factor
7	Radiographic changes

2.1.2 Prevalence

The prevalence of RA in Sweden has been estimated to 0.5-0.7% [8-10]. Studies of the prevalence in other northern Europe countries and most studies from north America

also suggest prevalence estimates between 0.5-1.0%, whereas somewhat lower estimates have been reported from southern Europe [11]. A higher prevalence of about 5% has been observed in some north American native populations [12, 13]. Prevalence studies outside Europe indicate variations between different ethnic populations with very low prevalence estimates in populations from sub-Saharan Africa. Some studies also indicate higher prevalence in urban than in rural areas although the pattern is somewhat inconsistent [13]. Since RA is a chronic disease, the prevalence increases with increasing age, and should thus (unless age-standardised) be higher in countries with long life-expectancies. The variations in prevalence among studies may thus be explained by variations in diseases assessment as well as age distributions across the studied populations. A declining prevalence has been reported in a few studies [14, 15].

2.1.3 Incidence

The peak incidence of RA is between the fourth and sixth decade and the incidence peaks in women about 10 years earlier than in men. The overall ratio between women and men is 3:1, but in postmenopausal women the incidence becomes similar to that of men. The incidence rates of RA vary between 20 and 50 cases per 100 000 per year in North America and northern Europe[11]. In a Swedish prospective study, the incidence was estimated to 24 per 100 000 in southern Sweden [16]. Studies of RA incidence from developing countries are lacking and there are relatively limited data on trends in incidence over time, but a few studies have suggested a falling incidence since the 1960s [15, 17-20]. There are also studies suggesting a shift towards higher age at disease onset [21, 22]. Together, the declining incidences and higher age at onset during the last decades may suggest the importance of birth cohort effects, or at least environmental exposures that have changed over time.

2.1.4 Disease subsets

RA is, as described above, a clinically heterogeneous disease. Despite decades of research, the cause of RA is unknown. RA is a complex genetic disease, where an interplay between several genes, several environmental factors and chance contribute to development of clinical manifest disease. As our understanding of RA pathogenesis has increased it is becoming more obvious that what, at a first glance, looks like one disease may in fact be a collection of many different diseases, or distinct subsets, each with different genetic and environmental risk factors and different pathways leading to disease.

Rheumatoid factor (RF) is detected in up to 80% of patients with RA and is classically used to divide RA into two subsets; RF positive and RF negative disease. In the late 1990s antibodies to citrullinated protein antigen (ACPA), sometimes referred to as anti-cyclic citrullinated peptide (CCP), were shown to be somewhat more specific for rheumatoid arthritis than RF, thus being more informative as diagnostic test for early RA [23, 24]. Antibodies to citrullinated protein antigen are present in about 60% of RA patients. Both RF and ACPA have a role as prognostic factors. A positive ACPA test appears to predict the development of severe RA and this predictive value complements that of RF [25]. Both ACPA and RF have also been shown to be detectable years before clinically overt disease is diagnosed, RF not so far back as ACPA, implicating an insidious change in the immune system during several years [26, 27].

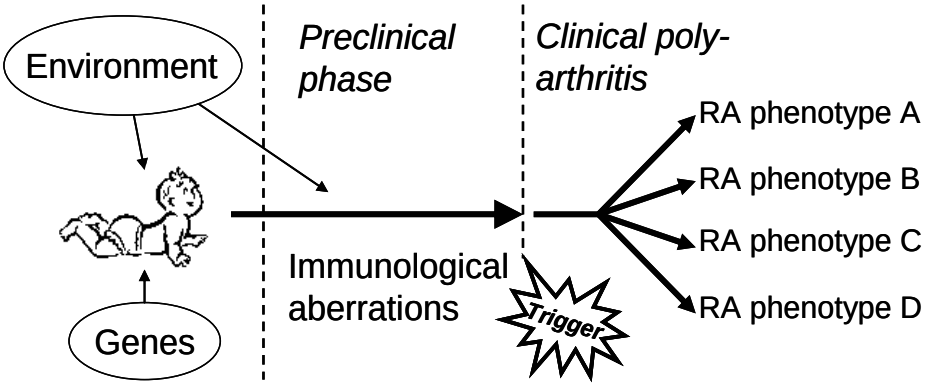

Figure 1 Schematic outline of RA development

2.1.5 Genetic predisposition

In twin studies, the concordance for RA among monozygotic twins has been estimated to approximately 15%, which is about four times that among dizygotic twins [28, 29]. From the same twin studies the heritability of RA, i.e. the relative contribution of genetic factors, has been estimated to be about 50-60% [30].
It has been known since the 1970s that alleles within the major histocompatibilty complex (MHC) region on chromosome 6 confer susceptibility to RA [31]. Subsequent studies showed that this risk is largely conferred to alleles within the human leukocyte antigen (HLA)-DRB1 locus sharing a conserved amino acid sequence, known as the "shared epitope" (SE) [32]. The HLA-DRB1 gene encodes the MHC class-II molecule which plays an important role in the presentation of antigens to the immune system. The association with HLA-DRB1 alleles has been consistently reported in many populations worldwide although the subtypes seem to differ between ethnic groups [33]. HLA-DRB1 genotypes have also been associated with more severe disease [34, 35]. The contribution of HLA-DRB1 to the overall heritability of RA has been estimated to about a third [36].
During the last years several other potential susceptibility genes outside the MHC region have been identified. There is strong evidence of an association with the R620W allele in the PTPN22 gene, coding for tyrosine phosphatase that has a role in T-cell and B-cell signaling, in patients of European ancestry [37-39]. For both HLA-DRB1 alleles and PTPN22 the association with RA is more strongly (or exclusively) associated with RF positive/ACPA positive RA [38, 40, 41]. Other risk alleles identified are TRAF1, STAT4 and PAD14 (for Asian populations) [42-44].

For ACPA negative disease an association with HLA DR3 has been observed [45, 46]. Recently there have also been indications of an association between the interferon regulatory 5 gene and ACPA negative RA, implicating a possible role for the interferon pathway. These findings further strengthens the idea that there are at least two distinct subsets of RA with different genetic and environmental risk factors and different pathogenetic pathways [47].

2.1.6 Environmental risk factors

Apart from the above, there are some methodological considerations when interpreting studies on RA etiology. Many studies are performed on RA patients with established disease rather than newly diagnosed RA patients. Studies may use self reported RA or physician diagnosed RA.
Although a significant portion of the risk for rheumatoid arthritis may be explained by environmental factors, few risk factors have been identified and the reproducibility has often been low. So far, smoking is the strongest known and best replicated environmental risk factor for RA. Together with other potenital risk factors of particular interest in this thesis (perinatal risk factors, chapter 3.1.4 and infections, chapter 4.1.1) smoking will be further discussed in chapter 5.2.1. Below, other risk factors for RA will be briefly described.

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Hormonal factors

The higher incidence of RA in women than in men suggests that hormonal factors may influence the risk of developing disease. Oral contraceptives have, although not consistently, been associated with a decreased risk of RA [48-50]. On the other hand, one study by Walitt et al reported no differences in the risk of developing RA, or the severity of RA, between a postmenopausal hormone therapy group and a placebo group [51]. In a study from the Iowa women cohort, former hormone replacement therapy was associated with an increased risk of RA [52]. The onset of disease is reduced during pregnancy, but increased in the first months post partum [53]. The reduced risk, and also amelioration of disease, during pregnancy have been suggested to be due to changes in the maternal immune system (a Th2 shift and/or increased number of regulatory T cells) [54]. Other mechanisms, such as the effect of prolactin and changes in the innate immunity, have been suggested to explain the increased incidence post partum and deterioration of disease during lactation [55, 56]. By contrast a few studies have found that long term breast-feeding is associated with a decreased risk of RA [48, 57, 58] although another small study showed an increased risk of RA in those who had breast-fed [59]. The long-term effects of pregnancy are somewhat unclear with some studies showing that women who are nulliparous have an increased risk of RA whereas others failed to show any association [57, 58, 60-62]**.** In a recent national Danish cohort study of reproductive factors, an increased risk of RA was observed for one-child mothers and young parents [63]. The risk of RA was also increased in women whose pregnancies were complicated by hyperemesis, gestational hypertension or pre-eclampsia.

Obesity, occupational exposure and socioeconomic status

There are inconsistent findings regarding obesity and risk of RA. Three out of five studies report a positive association and one study a borderline significant positive association among men [50, 61, 64-66].
Crystalline silica is found in rock, sand and soil. Prolonged or acute high exposure of silica can cause pulmonary inflammation and fibrosis, silicosis. Silica particles are deposited in the alveolar spaces in the lung where they are phagocytosed by alveolar macrophages. Occupational exposures to inhalation of silica and exposure to mineral oil have been found to increased the risk of RA [67-72]. The mechanisms by which silica can contribute to development of autoimmune disease are unclear, but a role of

activation and induction of apoptosis of alveolar macrophages has been suggested [73, 74].

Some studies indicate an inverse association between socioeconomic status, measured by education and occupational class, and risk of RA [66, 75-77].

Alcohol and dietary factors
Measuring dietary intake in epidemiological studies remains a problem and it is difficult to distinguish effects of various nutrients from each other. Another problem is to distinguish the effect of diet from that of other lifestyle factors. Alcohol consumption may decrease risk for RA [50, 65, 78]. Dietary factors have been studied in the context of RA progression, but also as risk factors for the development of RA. Coffee and tea consumption has been investigated but results are conflicting [50, 79-81] The role of vitamins has been studied, but also here findings are inconsistent [81-85]. Red meat consumption has been suggested to increase the risk of RA. One study showed an association between red meat and protein intake while another study could not confirm this finding [86, 87]. Recently, a somewhat decreased risk of RA among high-consumers of oily fish was suggested [88].

Other potential risk factors
One study by Symmons et al found an association between a history of blood transfusion and RA [64], whereas in two other studies no increased risk was observed [50, 89].

2.2 JUVENILE IDIOPATHIC ARTHRITIS

2.2.1 Clinical course and subsets

Juvenile idiopathic arthritis is a chronic arthropathy affecting children or adolescents who are under age 16. It is diagnosed by the presence of a chronic persistent arthritis of at least six weeks and other causes should be ruled out. Different classification criteria have been used to identify discrete clinical subsets. The current terminology, the International League of Association for Rheumatology (ILAR) classification criteria, was adopted in 1997 in order to achieve international consensus and due to the recognition that a true rheumatoid factor positive RA is infrequent in children under 16. The seven subsets of JIA include systemic onset, oligoarthritis, rheumatoid factor positive and negative polyarthritis, psoriatic arthritis, enthesitis related arthritis and other arthritis [90, 91]. The different subtypes of JIA further seem to represent different diseases with distinct clinical features and prognosis.

2.2.2 Incidence and prevalence

Epidemiological studies of JIA have been hampered by a lack of standardised criteria and case ascertainment, resulting in wide-ranging results. In a Nordic study the incidence of JIA, according to the ILAR classification criteria, was 15 per 100 000 children/year, with a variation between 7 and 21 per 100 000 children/year [92]. The reported world wide prevalence ranges from 70 to 400 per 100 000 [93]. Two studies

performed in Sweden reported a prevalence of 56 and 86 per 100 000 respectively [94, 95].

2.2.3 Risk factors

The etiology of JIA is poorly understood. So far, epidemiological studies have focused mainly on genetic aspects. The heterogeneity of the disease implies that there are different genetic and environmental risk factors that contribute to disease. Methodologically, the terminology and ascertainment of the disease make studies difficult to perform and interpret. When combining clinical distinct entities such as systemic JIA and oligoarticular JIA results become less easy to interpret. On the other hand stratifying for each subtype leads to loss of power which is crucial due to the low incidence of each subtype. So far, most studies have not distinguished between different subtypes and earlier studies vary in which criteria that have been used. Several associations between JIA and variants in the genes encoding HLA have been confirmed and replicated in independent cohorts [96]. A linkage of JIA to the HLA region was also found in a genome-wide scan study of 121 families [97]. Many non-HLA genes have been investigated, but only a few have been independently confirmed. These candidate genes include PTPN22, MIF and TNFA.

The literature on environmental risk factors for JIA is very scarce. Infection remains the most favoured hypothesis although evidence for a causal role is still lacking. Seasonal variation of systemic onset JIA has been investigated in a few studies but no clear association was found [98-100]. A Danish study of socioeconomic background showed an increased risk in children without siblings, children whose parents had a high income and children living in an urban areas [100]. Viral agents such as Parvovirus B19, Epstein-Barr virus, rubella, influenza A, Coxsackie, cytomegalovirus as well as bacterial agents have been suggested as triggers of JIA onset but the role of infections in the pathogenesis is still unclear [101-105]. There are ywo studies on other environmental factors, one showing that children with JIA were less likely to have been breast-fed than controls and one showing an increased risk for JIA in girls exposed to maternal smoking during pregnancy [106, 107]. Interestingly, maternal smoking during pregnancy has been shown to change the toll-like receptor (TLR) innate neonatal response [108].

2.3 INFLAMMATORY BOWEL DISEASE, SARCOIDOSIS AND MULTIPLE SCLEROSIS

2.3.1 Inflammatory bowel disease

Inflammatory bowel disease comprises two diseases entities, UC and CD, and tupically affects young adults. The incidence of UC and CD has increased during the last century. In the Nordic countries a current incidence of UC and CD of around 13/100 000 and 8/100 000 respectively have been reported [109]. Because of the relatively young peak incidence, its chronic nature and good prognosis, the prevalence of inflammatory bowel disease is high. The diagnosis of UC and CD is based on clinical symptoms, endoscopic and histologic findings [110]. Ulcerative colitis affects a varying portion of the colon and is characterised by continuous, diffuse mucosal inflammation with or without ulcerations. Crohn’s disease may affect any part of the gastrointestinal

tract. The inflammation is discontinuous, granulomatous and may be transmural. Although the overall prognosis is good, the clinical course is, like RA, characterised by periods of remissions and exacerbations. Inflammatory bowel disease, especially UC, is a risk factor for colorectal cancer. The etiology of inflammatory bowel disease remains unclear. Changes in incidence over time suggest the importance of environmental risk factors. As will be discussed in chapter 5.2.2 smoking is one of the few established risk factors for inflammatory bowel disease, although having different effects on the risk of UC and CD. Several other risk factors have been suggested: infectious agents, disruption or unbalance of the non-pathogenic intestinal flora, westernised diet rich in refined sugar and low in fiber, oral contraceptives, perinatal events and appendectomy [111]. Family studies on inflammatory bowel disease indicate a strong familial aggregation and several of inflammatory bowel disease susceptibility genes have been identified [112].

2.3.2 Sarcoidosis

Sarcoidosis is a granulomatous disease of unknown aetiology, with approximately 2000 new cases in Sweden yearly (incidence has been estimated to 24/100 000) [113]. The peak incidence is between 20 and 39 years of age. Although a systemic disease, it most commonly affects the lungs. Löfgren's syndrome, an acute presentation consisting of arthritis, erythema nodosum, and bilateral hilar adenopathy, occurs in 9 to 34% of Scandinavian patients, but the phenotype varies somewhat between ethnic groups. The diagnosis is based on clinical, radiological and sometimes histological findings. The majority of patients with acute sarcoidosis recover, but some develop chronic disease with fibrosis and eventually respiratory failure [114]. The risk of sarcoidosis depends on both genetic and environmental exposure. HLA-DQB1 and HLA-DRB1 have been consistently associated with sarcoidosis [115]. The search for environmental factors has focused on inhaled agents. Several potential risk factors for sarcoidosis have been reported such as insecticides, moldy environments and different occupational exposures. Suggested infective triggers such as mycobacteria and Propriumbacterium acnes are yet unconfirmed [116].

2.3.3 Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterised by repeated subacute episodes of nervous system symptoms and signs of demyelination followed by remission. It is a disease affecting young adults between 20 and 40 years of age. The incidence of MS is about twice as high in women as in men and increases with the distance from the equator. Recently though, a systematic review indicated an attenuation of the latitude gradient over the last 25 years and an increase in the female to male ratio [117]. In Sweden, the incidence has been estimated to around 5/100 000 [118]. The diagnosis is based on clinical symptoms and findings on clinical, laboratory and radiological examinations [119]. The strongest known risk factor for MS is family history. Genetic studies have identified associations with HLA alleles (HLA-DR1501) within the MHC region [120]. Studies on geographical distribution of MS and changes in risks among migrants have led to several hypotheses involving environmental risk factors. One major hypothesis is the role of sunlight exposure and D-vitamin deficiency. Numerous studies have investigated the amount of sunlight exposure and risk of MS, but have been hampered by the possibility of reversed

causality, i.e. that MS itself induces changes in lifestyle [121]. An association between D-vitamin deficiency and MS has been supported by a prospective study of the Nurses Health Study Cohort and a US case control study on levels of D-vitamin available from the Department of Defense Serum Repository [122, 123]. Epstein-Barr virus infection is another risk factor that has been suggested, but the role of Epstein-Barr virus infection remains unclear. Smoking has in several studied been associated with the risk of MS, but the role of smoking will be further discussed in chapter 5.2.2.

3 EARLY LIFE EXPOSURE AND RISK OF RA

Observations such as the presence of ACPA as well as RF several years before clinical onset of RA disease, as discussed above, suggest that the pathogenesis of RA is protracted and includes a role for specific as well as unspecific immune reactions, and thus that critical environmental exposures may act much earlier than previously thought. Additionally, a decreasing incidence of RA in combination with a shift towards higher age at diagnosis suggests a birth cohort effect, in turn implicating that risk factors acting early in life might be of importance.

3.1.1 Developmental origins of health and disease (DOHaD)

Already in the 1930s Kermack et al came up with the hypothesis that early life environment is associated with later mortality [124]. Through studies on the relationship between infant mortality and mortality rates from arteriosclerotic heart disease in the 1970s, Anders Forsdahl, Norway, suggested that poverty in childhood and adolescence followed by prosperity is a risk factor for arteriosclerotic heart disease [125]. In 1980s, Barker and his colleagues in UK could confirm these findings, which led to the hypothesis that malnutrion in utero permanently changes the body's structure, function and metabolism in ways that increase the risk of coronary heart disease later in life, commonly referred to as "the fetal origin hypothesis" or "Barker hypothesis"[126]. Since then, numerous epidemiological and clinical studies have related early life events to risk of coronary heart disease, hypertension, stroke and type 2 diabetes [127]. Later this hypothesis was developed further to encompass also the postnatal period and it is now referred to as "the developmental origins of health and disease" (DOHaD). Early life events, occurring from conception to infancy, acting through the process of developmental plasticity, alter development of the organism to such an extent that it affects its capacity to cope with the environment later in life [128, 129]. The mechanisms behind this hypothesis are unclear and presumably different for different diseases, but may partly be explained by epigenetics, heritable changes in gene expression potential not caused by changes in the DNA sequence [130, 131].

3.1.2 An overview of the immune system

The physiological role of the immune system is to protect us from infections and other foreign substances. However, the immune system is also capable of reacting not only against components of microbes but also against other proteins and polysaccharides as well as chemicals causing tissue damage regardless of the consequence of such a reaction.

The immune system is divided into the innate and the adaptive immune system. The innate immune system provides the initial defense against microbes. It consists of physical and chemical barriers such as the skin and epithelial lining of the gastrointestinal tract, immune cells such as phagocytic cells and natural killer cells, proteins in the complement system and other mediators of inflammation, including cytokines. Components of the innate immunity recognize structures (pathogen associated molecular patterns, PAMPs) that are characteristic of microbial pathogens, but shared by classes of microbes, efficiently distinguishing microbes from self-

molecules. Receptors that bind these structures, pattern recognition receptors, include for example TLRs. However, the innate immune system can also recognize stressed or injured host cells which often express molecules not found in great extent in healthy cells.

The adaptive immune system on the other hand is very specific and has a capacity to distinguish between different, even closely related microbes and molecules and has the ability to remember and respond more aggressively to repeated exposure. The cells of the adaptive immune system are present as circulating cells in the blood and lymph, but also in lymphoid organs and as scattered cells in virtually all tissues.
The immune responses are specific to distinct antigens and the total number of antigenic specificities is extremely large, estimated to about 10^7 to 10^9. An antigen can be almost any kind of biologic molecule including sugars, lipids, hormones, phospholipids, nuclear acids, proteins and peptides. The normal immune system recognizes, responds to and eliminates foreign antigens but do not react to the individual's own antigenic substances. This "tolerance" is maintained by several mechanisms. Disruption of self-tolerance leads to immune responses against self antigens and may result in autoimmune diseases.

Lymphocytes, antigen presenting cells (APC) and effector cells are the principle cells of the adaptive immune system. There are different subsets of lymphocytes of which two major groups are: T-lymphocytes and B-lymphocytes. T-lymphocytes are further divided into helper T lymphocytes (Th cells, activating other cells), cytotoxic T lymphocytes (CTL, killing cells infected with microbes and tumor cells) and regulatory T-lymphocytes (Treg, regulates the function of other T-cells and helps maintain self-tolerance). There are two major subsets of Th cells, Th 1 and Th 2. Th 1 cells mainly produce interferon-γ (INF- γ) and Th 2 cells produce interleukin-4 (IL-4) and IL-5. The role of Th 1 cells is to recognize microbial antigens and activate phagocytes to destroy the ingested microbes. Th 2 cells are responsible for the immune response to helminths and allergens. Each of these subsets amplifies itself and cross regulates the reciprocal subset so that once an immune response develops along one pathway it becomes increasingly polarized in that direction. A third subset of Th cells, Th 17 cells, secreting IL-17, has recently been identified.

B-lymphocytes have antigen presenting ability but most importantly can be activated to antibody (or immunoglobulin) producing plasma cells. Important differences between T and B lymphocytes include the fact that T lymphocytes can only recognize peptides displayed on other cells as antigens, whereas B lymphocytes can recognize soluble antigens as well as cell-associated antigens of different kinds as mentioned above. Specialised proteins encoded by genes in the MHC region are needed to present cell-associated antigens. MHC class I molecules present antigens to CTL and MHC class II molecules present antigens to Th cells.
The adaptive immune system is further divided into two subtypes: the cellular and the humoral immune system. Cell-mediated immunity is mediated by T lymphocytes and serves as the defense mechanism against microbes that survive within phagocytes or infect nonphagocytic cells, whereas humoral immunity is mediated by antibodies produced by B-lymphocytes and is the principal defense against extracellular microbes and their toxins.

3.1.3 Development of the immune system

The immune system mainly develops during fetal and early life but remains plastic throughout life. Pluripotent erythroid and granulomacrophage progenitors can be detected in the circulation from 4 weeks of gestation. They migrate to the liver which is the major site of haematopoesis during the first and second trimester of gestation. The thymus (where maturation of T cells takes place) and spleen are seeded from the liver. Stem cells are detectable in the bone marrow (where maturation and of B cells takes place) at the end of the first trimester. The immune cells during fetal life are different from those in adults both morphologically expressing other surface proteins (CD molecules) and functionally with respect to production of cytokines and immunoglobulins.

Our current understanding of the postnatal maturation of the immune system is limited, but it is clear that the maturation of the immune system continues throughout childhood and early adolescence. In the fetal immune system Th 1 is down regulated. Postnatally, the principal stimuli of maturation of the immune system are signals from the microbial environment, particularly the commensal microflora of the gastrointestinal tract, but also infections in the gastrointestinal and respiratory tracts [132]. The focus of this maturation process is thought to be up-regulation the Th 1 response. Environmental factors acting during the development and maturation of the immune system in early life, including fetal life, may have an impact on the function of the immune system later in life.

3.1.4 Early life exposure and later risk of RA and other autoimmune/inflammatory diseases

In contrast to the little-known effects of early life exposures on the risk of later rheumatic inflammatory diseases, the role(s) of early life factors has been extensively studied with respect to other types of autoimmune/inflammatory/allergic diseases. Apart from exposure to microbes and infections, pre- and perinatal factors such as maternal smoking and diet, prematurity and maternal stress have been suggested to influence the risk of childhood asthma and atopy [133, 134]. Intrauterine growth pattern has been associated with coeliac disease and childhood onset type 1 diabetes [135-139]. Additionally, several other perinatal events such as blood group incompatibility, gestational infections, preeclampsia and breast feeding have been identified as risk factors for type 1 diabetes [140]. As shown in studies on the timing of introduction of gluten, wheat, oat and fish in the infant diet and the risk of coeliac disease, wheat allergy and allergic rhinits it has become clear that the timing of environmental exposure may be just as important as the exposure per se [141-144].

Early life exposures and the risk of RA have been investigated in some studies. In a small case control study by Jacobsson et al. and in a US study based on the Nurses Health Study, high birth weight (≥4000 g and ≥4500 g) was associated with an increased risk of RA [145, 146]. Jacobsson et al also found an inverse association with initiation of breast feeding during inpatient care after delivery. A recent study from the Nurses Health Study could not confirm an association between being breastfed and RA,

nor did they find an association between preterm birth and RA [147]. In a UK study, rheumatoid factor positivity in adult women was dependent on their childhood living conditions[148] and in another study a strong inverse association between duration of breastfeeding, > 3 months, and rheumatoid factor positivity among a subset of children who were negative for HLA-DR4 was observed [149].

Early life exposures and later disease risk has also been assessed in a few studies of other rheumatic diseases. A relationship between birth weight and primary Sjögren's syndrome as well as systemic lupus erythematosus have been reported, although not consistently for the latter disease [150-152].

4 INFECTIONS AND LATER RISK OF RA

Although infections and their role in the pathogenesis of RA have been extensively studied during several decades, focus has been at the time around the onset of disease. No studies have investigated the role of infections during childhood and adolescence and later risk of RA. In the context of other autoimmune/inflammatory diseases, infections during childhood and later disease risk have been investigated. In type 1 diabetes, celiac disease, and inflammatory bowel disease, associations with the pattern of childhood infections have been suggested [136, 153, 154]. A reduced risk of allergy, atopy and asthma has been related to exposure to microbes (rather than overt infections) whereas viral and respiratory tract infections have been associated with increased risk of asthma [155].

4.1.1 Studies on the role of infections as triggers of rheumatoid arthritis

For many years, research was focused (and some still are) on finding *the* single microbial agent causing RA. As it is now clear that the etiology of RA is multifactorial, the finding of a single pathogen that causes RA is improbable, though possible for a subset of RA. Furthermore, disease onset may occur months or years after the potential initiating infectious agent is cleared by the immune system making it difficult to assess from a clinical point of view. Additionally, even when a specific tentative microorganism has been identified, its role in the pathogenesis is difficult to define. It may be a nonspecific finding or it may be a secondary event occurring in an already inflammatory environment.

A large number of infectious agents have been investigated such as Parvovirus B19, Epstein-Barr virus, retroviruses, Alphaviruses, Mycobacteria, Mycoplasmas, Proteus mirabilis and Escherichia coli (E.coli) [156-163]. Different study designs have been used including serological evaluation of patients with newly diagnosed as well as established RA, in vitro culture of bacteria from blood, urine and synovial fluid as well as analysis of bacterial DNA and bacterial proteins in synovial fluid or tissue [157, 164, 165]. Most of the studies have been performed on hospital-based patients, often with established RA, with a focus on only one or a few candidate microbes. Söderlin et al. did a population-based systematic survey of the infectious background of patients with early synovitis (duration of symptoms <3 months). The occurrence of recent infection was evaluated through medical history and serology. Among all patients (n=71), 45% had evidence of recent infection. Of the patients with early synovitis, 15 (21%) experienced RA, and 2 (13%) of these had serologic evidence of recent infection [166]. Evidence for a causal role of the investigated infections in the pathogenesis of RA is, however, still lacking.

4.1.2 Potential mechanisms in the pathogenesis of autoimmune diseases

Infectious agents may contribute to the pathogenesis of RA in many ways through different mechanisms affecting both the innate and adaptive immune system.

Pathogens trigger the innate immune cells through pattern recognition receptors, such as TLRs, which increases the antigen presenting capacity, the expression of co-stimulatory molecules and production of cytokines. The microbial antigens as well as inflammatory signals drive the T-cell and B-cell activation. In this scenario pathogens may act as *adjuvants* for the immune response activating autoreactive T-cells.

T-cells can respond to different peptides and T-cells receptors (TCR) may cross react with different peptide-MHC complexes as long as they have similar shape and charge distribution. This may result in T-cells cross reactive with self antigens. Similarly, monoclonal antibodies have been found to recognize both microbial and self antigens. When a T- or B-cell receptor recognises a microbial peptide that is structurally similar to a self peptide, the immune response may spread to tissues that present cross reactive self peptides, resulting in autoimmunity through *molecular mimicry*.

In the inflammatory milieu of a pathogenic infection, APCs present self antigen, obtained following tissue destruction, to autoreactive T-cells. This process, known as *bystander activation*, facilitates the maintenance of inflammation even when the pathogen is cleared. In addition, autoreactive T-cell or B-cell responses induced by a single peptide (or epitope) can spread to include other peptides (or epitopes) in the same autoantigen or in other self antigens that are released after T- or B-cell mediated bystander activation. This is called *epitope spreading* and can be beneficial in the context of a pathogen or tumor defense because the pathogen or tumor cannot easily escape immune control [167].

Antibodies directed to self antigens, so called autoantibodies, can be induced by infections. Infectious agents that may induce production of autoantibodies such as RF are parvovirus B19, hepatitis B and C, alphaviruses and mycoplasmas, although the pathogenetic role of these antibodies in RA is unclear. Another example is Epstein-Barr virus. Epstein-Barr virus infects B-cells, persists in a latent form in memory B-cells, is a potent stimulator of polyclonal B-cell proliferation and can induce proliferation of autoantibody-producing B-cells. There are also examples of molecular resemblance between Epstein-Barr virus and self antigens leading to cross reactivity (molecular mimicry) where B-cells and antibodies react to self antigens. This has also been suggested for Mycobacteria. Epstein-Barr virus antigens can undergo posttranslational citrullination, becoming targets for anti-CCP possibly inducing immune response against citrullinated human proteins [157].

T-cell tolerance is developed during maturation of T-cells in the thymus and is maintained through anergy, deletion and suppression by Tregs in the periphery. As described above, autoreactive T-cells may be induced by molecular mimicry between foreign and self antigens. One hypothesis is that the target epitope for mimicry may be the disease associated HLA molecules themselves. In the context of RA, Epstein-Barr virus has been suggested to induce cross reactive T-cells, but this has also been suggested for other viruses and bacteria such as E.coli [156, 157, 162, 168, 169].

The process where infections may lead to an autoimmune/inflammatory disease is definitely more complex than presented above. For example, even if autoreactive T-cells are present this may not lead to autoimmune disease. Its development may instead

depend on various coincidental events including the number of autoreactive T-cells, the avidity and affinity of these cells and the presence of innate inflammatory signals required for these T-cells to gain pathogenic phenotype. These events may not need to happen at the same time. The contribution of microbial infection to autoimmunity/chronic inflammatory disease should be regarded as a process involving many pathways rather than a single event involving a single mechanism. Further, it may be the other way around. A predisposition to autoreactive immune responses might affect the development of antiviral immune responses.

5 THE INFLAMMATORY REFLEX, TOBACCO AND RISK OF CHRONIC INFLAMMATORY DISEASES

5.1.1 The inflammatory reflex and its potential role in chronic inflammation

The "inflammatory reflex", acting via the vagus nerve, represents a physiological pathway regulating the immune response.
Historically, the role of the immune response to pathogens was believed to solely eradicate the invader. The development of shock, tissue injury, circulatory collapse etc was attributed to a direct effect of the pathogenic toxins. During the 1980s, studies of cytokines lead to the cytokine theory of disease, the concept that overproduction of cytokines by the immune system can cause the signs, symptoms and damaging effects of disease. For example, tumor necrosis factor α (TNFα), a proinflammatory cytokine, was shown to have the capacity to cause fever and localised inflammation and to mediate septic shock caused by infection with Gram-negative bacteria [170]. Parallel research in the pathogenesis of RA showed a key role of TNFα in RA inflammation [171, 172] and subsequent studies revealed therapeutic success with TNFα blockade [173].

The vagus nerve is part of the parasympathetic system, is finely branched and is composed of a sensory (afferent) and output (efferent) part. It is the longest of the cranial nerves and innervates most peripheral organs in humans.
In a series of experimental studies, Tracey et al. have shown the existence, function, and importance, of the "inflammatory reflex", [174-177] that comprises a fast and efficient mechanism for neural inhibition of systemic inflammation, consisting of an afferent arm sensing inflammation and an efferent arm, the cholinergic anti-inflammatory pathway, inhibiting innate immune response. In experimentally induced endotoxaemia in rats, direct electrical stimulation of the vagus nerve significantly attenuates serum levels of TNF, hepatic TNF-synthesis, and prevents the development of shock [174-176]. In other experimental models, vagus nerve stimulation has been shown to reduce also peripheral inflammation [175]. Conversely and in the same animal experiments, vagotomy has been associated with an exacerbated TNF-response during endotoxaemia, sensitisation to and inability to control inflammatory stimuli [174-176]. Recent studies indicate that the principal components for cytokine suppression by the vagus nerve converge in the spleen and spleen vagal innervation is crucial in this context [178-180]. Furthermore, cholinergic signals to the spleen results in downregulation of leukocyte trafficking and migration to peripheral inflammatory sites [181].

Importantly, specific activation of the α7 nicotinic acetylcholine receptor (α7nAChR) is essential in the inflammatory reflex, and knockout mice for this receptor have no immunosuppressive effect of vagus nerve stimulation [182]. The intracellular mechanisms involved in a7 mediated downregulation of pro-inflammatory cytokines on macrophages and other immune cells are complex, but an important step is the inhibition of the nuclear activity of NF-κB [182-184]. As a potent agonist of the α7nAChR, nicotine may exert strong anti-inflammatory effects, termed the "nicotinic

anti-inflammatory pathway"[185]. Importance of cholinergic regulation in arthritis has been confirmed in experimental arthritis, where specific a7 agonists suppress joint inflammation [186] and the a7 receptor has also been detected in RA joints in mainly fibroblasts and macrophages [187]. Moreover, RA patients have decreased vagus activity compared to healthy individuals, and RA vagus activity is associated with increased serum HMGB1 levels [188].
Based on these data it has been discussed if vagus nerve function may be of importance not only for potent regulation of acute inflammation, but also for the exacerbation and progression of RA and other chronic inflammatory diseases.
Decreased activity in the cholinergic anti-inflammatory pathway might result in suppressed ability to counteract excessive cytokine expression, and thereby facilitate development of autoimmune reactions, which in genetic susceptible individuals may subsequently lead to development of chronic conditions such as RA and IBD.

Studying the impact of vagus nerve activity as a risk factor for RA in humans is thereby of interest, not only for detection of a possibly altered risk for the disease, but also to increase the knowledge of the general mechanisms of this pathway in a human setting. In addition, studying the effect of nicotine, which, as described above, is a potent agonist of the α7nAChR, offers a possibility to indirectly study the role of the inflammatory reflex and cholinergic immune regulation in the context of chronic inflammatory diseases.

5.1.2 Nicotine and the immune system

Animal studies suggest that nicotine has several immunomodulatory effects with the capacity to inhibit both the innate and the adaptive immune response, but the role of nicotine in human disease remains debated [189].

Nicotine exerts its effects by activating nAChRs, which are found in the autonomic ganglia, central nervous system, neuromuscular junctions and adrenal medulla, but also on immune cells such as monocytes, dendritic cells and microglial cells [182, 190]. Additionally they have been described in the bronchial epithelium and the small and large intestine [191].

Animals treated chronically with nicotine show a significant loss of antibody response and T-cell proliferation [192, 193]. A critical intracellular pathway involved in the production of pro-inflammatory cytokines in innate immune cells is the NF-κB pathway, briefly mentioned above [194]. Nicotine inhibits the NF-κB pathway, and suppresses HMGB1 release from human monocytes and macrophages through the α7nAChR [195, 196]. This has been confirmed in other cell types such as microvascular endothelial cells and microglia cells, indicating that the nicotinic anti-inflammatory pathway may not be limited to cells of monocyte lineage [197, 198]. Nicotine has also been shown to affect alveolar macrophage and dendritic cell responses, although the results concerning dendritic cells are somewhat contradictive [195, 199-201]. Further, the acute effects of nicotine on the immune system seem to be mediated via the activation of the hypothalamic-pituitary-adrenal axis leading to increased levels of glucocorticoids [202]. Taken together these findings suggest that the

impact of nicotine on the immune response is complex but supports a possible protective anti-inflammatory effect.
Although these potential protective effects of nicotine have been demonstrated in many animal models its effect in humans needs to be clarified. For example nicotine is assumed to mediate the positive effect of smoking on ulcerative colitis (UC), but although transdermal nicotine treatment has been beneficial in active UC in some studies, results are inconsistent across studies [203].
In one study the effect of nicotine patch on human in vivo response to bacterial endotoxin (lipopolysaccharide, LPS) in 11 healthy men was investigated. Subjects receiving nicotine patch experienced significantly lower temperature response as well as attenuated cardiovascular response. There was no significant difference in circulating TNFα, IL-6 or IL-8, but the results suggested a faster return to baseline value in nicotine-treated subjects. On the other hand nicotine-treated subjects had increased IL-10 response and increased levels of cortisol compared to placebo-treated subject. Thus in this model of LPS induced inflammation, nicotine exposure seemed to alter the inflammatory response and promote a more anti-inflammatory phenotype.

Smoking, an established risk factor for chronic inflammatory diseases which will be further discussed below (chapter 5.2.2), as well as use of moist snuff, lead to exposure to nicotine. Whether the strong associations between cigarette smoking and chronic inflammation is borne out by nicotine, or by other constituents of inhaled tobacco smoke is unclear, as are the risks with smokeless tobacco that contains nicotine but does not lead to airway exposure to other inhaled substances of tobacco smoke. Comparing risks associated with smoking and use of moist snuff thus gives a possibility to disentangle the role of nicotine from the effect of other inhaled components of tobacco smoke in the development of chronic inflammation.

5.1.3 Smoking vs. moist snuff

Since the 1970s the tobacco using habits in Sweden have gone through a transition. While tobacco smoking has decreased, the use of Swedish moist snuff, “snus”, has increased among both men and women. Today, approximately 20% of Swedish adult male population are daily users of moist snuff although the use among women is still uncommon (3%). Approximately 11% of Swedish adult men are daily smokers [204]. The proportion of users of moist snuff is largest in the age group 30-44 years of age, while the proportion of daily smokers is largest in the age group 45-64 years of age [204].

The major component in tobacco is nicotine, a highly addictive alkaloid. Tobacco smokers are exposed to nicotine, but also to more than 4 500 other chemicals. Major health hazards of tobacco smoke are well known, but the role of nicotine is still unclear. Users of moist snuff are exposed to similar or higher concentrations of nicotine [205]. The absorption of nicotine through the oral mucosa is slower than when tobacco is

Swedish moist snuff

smoked, leading to higher levels among snuff users during longer period of time [206]. Swedish moist snuff is pasteurised, which leads to lower concentrations of nitrite and tobacco specific nitrosamines compared to American moist snuff, which is fermented [207]. The different routes of tobacco administration (through inhalation vs. through the oral mucosa) between smokers and snuff users may have an impact on the effects of nicotine and other tobacco constituents on the immune system.

5.1.4 Smoking and the immune system

Health consequences of cigarette smoking are well known. Smoking is considered to have a crucial role in the pathogenesis of many diseases. It has been speculated that many of the health consequences of chronic inhalation of cigarette smoke are due to its effects on the immune system.
Major effects of cigarette smoking in the lungs are seen, but also systemic effects. The consequences of cigarette smoking depend not only of the constituents in the tobacco smoke, but on the duration and intensity of smoking, genetic predisposition and other environmental factors. Cigarette smoke is composed of two phases, the vapour phase and the particulate phase. Chronic exposure to the vapour phase does not seem to suppress the immune system, which indicates that one or more components of the particulate phase are immunosuppressive. Nicotine is mainly associated with the particulate phase, as is tar, aromatic hydrocarbons, phenol and cresol.

Bronchial epithelium serves not only as a structural barrier but also possesses a functional role in the defense against foreign agents. Cigarette smoke increases the permeability and the excretory function of epithelial cells leading to enhanced levels of cytokines and adhesion molecules, in turn resulting in augmentation of inflammatory cells [208]. Cigarette smoking increases the number of alveolar macrophages (AM). These cells express and secrete increased levels of enzymes which may damage connective tissue and parenchymal cells of the lungs. On the other hand AM in smokers have a decreased antigen presenting capacity and a decreased ability to phagocytose and kill bacteria [209-212]. The mean life span of macrophages is prolonged in smokers, but cigarette smoke and oxidative stress enhance the apoptosis of AM [213]. Cigarette smoke can also induce apoptosis of bronchial epithelial cells and the augmentation of apoptotic material may cause impaired phagocytosis, necrosis and persistent inflammation. Bronchoalveolar lavage (BAL) fluid and bronchial biopsies taken from smokers are characterized by increased CTL (CD8+ T-cells) and a decreased CD4+/CD8+ ratio, which contributes to the destructive effects of cigarette smoke on lung tissue.

A well documented effect of cigarette smoking in humans is leukocytosis, although the function of these cells is reduced and the clinical effect unclear [189, 214]. Furthermore, in the context of cardiovascular disease, an elevation of inflammatory markers such as high sensitive CRP and IL-6 has been observed [215].

5.2 SMOKING AND CHRONIC INFLAMMATORY DISEASES

5.2.1 Smoking and rheumatoid arthritis

Smoking is the most established environmental risk factor for developing RA. The first study showing an association between hospitalisation due to RA and cigarette smoking was published in 1987 by Vessey et al [216]. Since then numerous case-control and cohort studies have confirmed this association [217]. The risk of RA increases with smoking intensity and duration and seems to be somewhat stronger in men than in women [218, 219]. The risk persists for many years after smoking cessation, but attenuates gradually [218-220]. Smoking has been shown to be a risk factor for the RF positive subset of RA and recently also for the ACPA positive subset (which overlap to a great extent), but to have minor effect on the autoantibody negative subset of RA [50, 76, 219, 221]. The role of long term passive smoke exposure is unclear and so far has only been evaluated in one study where no association was found [218]. In a study mentioned above, girls exposed to maternal smoking during gestation had an increased risk of juvenile idiopathic arthritis in the first 7 years of life [106].

As mentioned earlier, individuals who carry the SE have an increased risk of ACPA positive RA. Individuals carrying double SE alleles have a higher risk compared to individuals carrying a single allele. Recently, a gene-environment interaction between smoking and shared epitope has been suggested. In a Swedish study, the risk of developing ACPA positive RA for an individual who smoked and carried two copies of the shared epitope was 21-fold higher compared to non-smokers who did not carry the shared epitope [76, 221]. An interaction has also been observed in other European cohorts [40, 222-224].

The mechanisms behind the influence of smoking on RA remain unclear as does the very smoke component(s) responsible for these mechanisms. In a study by Klareskog et al, citrullinated peptides were detected in BAL cells in smokers but not in non smokers [221]. These findings led to the hypothesis that smoking can cause citrullination in the lung. This hypothesis is supported by the findings of higher expression of peptidylarginin deaminase (PAD)2 enzyme, which causes citrullination, in BAL cells and bronchial mucosal biopsy of healthy smokers than healthy non smokers [225]. Other possible inhaled environmental risk factors for RA such as dust from silica and charcoal could contribute to disease in the same manner. Another study by Bongartz et al. could however not confirm these findings [226], but found that citrullinated peptides were present to the same degree in lung specimens from RA patients with interstitial pneumonitis as in patients with idiopathic interstitial pneumonitis, but not in controls and there was no association with smoking habits. They also showed the presence of citrullinated peptides in rheumatic nodules, which supports the hypothesis that citrullination is an inflammatory related event [227].

5.2.2 Smoking and other chronic inflammatory diseases

Smoking is an established risk factor for CD, but has in some studies been associated with a somewhat reduced risk of UC whereas ex-smokers have an increased risk also for UC [228]. Smoking cessation has been shown to deteriorate the course of UC but improve the course of CD. As mentioned earlier the beneficial effect of smoking on UC

is assumed to be due to the anti-inflammatory effect of nicotine. Based on both animal and human studies, the opposite effect or lack of beneficial effect in CD has been suggested to be due to different effects on nicotine on the small and large intestine [229] or an impaired macrophage response to intestinal bacteria [199, 230]. The negative effect of smoking on CD might on the other hand be mediated through mechanisms independent of nicotine.

In a recent, large study on environmental risk factors in sarcoidosis, tobacco smoking was confirmed to decrease the risk for disease [231], consistent with previous studies [232, 233]. Other pulmonary granulomatous disorders such as hypersensitivity pneumonitis also seem to be less common among smokers [234]. In experimental studies on mouse and cell line models of hypersensitivity pneumonitis, nicotine was shown to reduce the BAL cellular response, the production of inflammatory cytokines and the extent of inflammation as analysed in biopsy samples [235]. As mentioned above, an influence of smoke on BAL cellular composition has been noted also in humans, e.g. an increased frequency of CD8+ T cells, resulting in a reduced CD4/CD8 ratio [233]. These immunomodulatory effects by nicotine have been suggested to relate to the reduced frequency of pulmonary granulomatous disease among smokers [236].

Several studies have shown an increased risk for MS among smokers [237-239] and a faster transmission from a relapsing-remitting clinical course to a secondary progressive course has been indicated [240, 241] Several possible mechanisms have been discussed including the effect of nitric oxide, chronic cyanide intoxication, the effect of nicotine on the blood brain barrier, and smoking mediated increased frequency and persistency of infections, but the relevance of these have to be established (see discussion in ref [237, 238]).

5.2.3 Use of moist snuff and chronic inflammatory diseases

Little is known about use of moist snuff and chronic inflammatory diseases. One recently published study by Hedström et al investigated tobacco smoking, use of moist snuff and the risk of MS [242]. They could confirm the increased risk of MS in smokers, however there was no association between use of moist snuff and the risk of MS indicating that non-nicotine components of tobacco smoking may be responsible for the increased risk of developing MS among smokers. In contrast they found a decreased risk (RR=0.3 95%CI 0.1-0.8) of MS among long term snuff users (>15 years).
Only one more study has investigated the relationship between chronic inflammatory disease and use of moist snuff. In a small retrospective case control study by Persson et al. in 1993, the association between use of moist snuff and inflammatory bowel disease was assessed among 152 prevalent cases with CD, 145 prevalent cases with UC and 305 controls [243]. Exposure information on snuff use was obtained by questionnaires. Use of moist snuff in never smokers was not associated with CD or UC, but an increased OR was observed for use of moist snuff and smoking in combination and the elevated OR remained also after adjustment for smoking in multivariate analysis. The study design had, as described above, several limitations and the results were based on small exposed numbers.

6 AIMS

The overall aim of this thesis was to broaden our understanding of the etiology of RA, in particular to explore the importance of the inflammatory reflex, childhood infections and early life exposures as risk factors for RA.

The specific aims were:

- To assess the role of birth characteristics and to explore the role of early life infections on the risk of developing RA.
- To confirm, in a larger study population, the role of infections during early childhood on the risk of developing RA and to extend the assessment of infections to encompass infections throughout childhood and adolescence.
- To explore whether surgical vagotomy in humans would affect the risk of developing RA.
- To assess and compare the effects of smoking and use of moist snuff respectively, on the risk of developing RA and other chronic inflammatory diseases.

7 METHODS

7.1 SETTING

Sweden has a long tradition of holding registers of the population. Already in 1749 a nationwide reporting system of causes of death was introduced [244]. Today two national agencies, Statistics Sweden and the National Board on Health and Welfare, are responsible for the nationwide Swedish national registers on demographics, morbidity and mortality. Since 1947 unique national registration numbers (NRN) containing birth date and gender have been assigned to all residents of Sweden. All Swedish national registers use the NRNs, which facilitates cross-registry linkages [245]. The Swedish health care system is public and tax funded making it equally accessible to all Swedish residents. There are also geographic referral patterns to specialist care. These circumstances all contribute to the exceptional possibilities for epidemiological research in Sweden using registers.

7.2 DATA SOURCES USED IN THIS THESIS

7.2.1 National health / demographic registers

The Swedish Inpatient Register or the Swedish Hospital Discharge Register
In English literature, this register has been referred to as both The Swedish Inpatient Register and The Swedish Hospital Discharged Register. It is a population-based register that contains information on individual discharges from Swedish inpatient care county-wise since 1964, nationwide since 1987. For each hospitalisation - defined as at least one overnight hospital stay - the NRN, one main diagnosis and up to seven contributory medical diagnoses, codes for surgical procedures, dates of admission and discharge, department and hospital are recorded. Medical diagnoses are coded according to the International Classification of diseases (ICD) versions 7-10. Validation studies have shown a near 100% completeness of the entire register. The validity of the correctness of the discharge diagnoses vary from one diagnosis to another. Studies indicate an overall specificity of 85% or above [246]. For example, the diagnostic accuracy of myocardial infarction has been evaluated and found to be almost 100%[247]. With respect to the RA-diagnosis, validations against the ACR-criteria suggest high diagnostic accuracy. In a study by Baecklund et al. the RA diagnosis was manually validated through scrutinising the medical records of 931 cases. Nine percent did not fulfill the ACR criteria [248]. With regards to inflammatory bowel disease the diagnostic validity seems to be somewhat lower, but still 85-90% of the discharges coded as UC or CD were found to be correct [249].

The Cause of Death Register
Statistics of causes of deaths have been published annually since 1911. The Cause of Death Register was set up in a computerised form in 1952 and includes the date of death, main and contributory cause of death (coded according to the ICD), and the NRN for all residents deceased during each year. The coverage is close to 100% and information on the cause of death is missing for less than 0.5% of the deaths.

The Register of Population and Population Changes
Since 1968, official Swedish census data has been compiled in the Register of Population and Population Changes. All Swedish residents alive in Sweden at the end of each year are included. The register contains the NRN, name, parish, community and county of domicile, civil status and information on dates of immigration and emigration.

The Swedish Medical Birth Register
The Swedish Medical Birth Register is a register covering nearly all births (98%) in Sweden since 1973 [250, 251]. The information is based on standardised medical records used by all antenatal clinics and delivery units in Sweden. Maternal characteristics recorded in the register include maternal age, information on previous pregnancies, diseases during pregnancy, smoking habits (since 1983) and family situation in early pregnancy. The register also contains information on delivery unit, mode of delivery and complications during pregnancy and delivery. Information about the infant includes NRN, whether stillborn or alive, single or multiple birth, birth weight and length, gestational age, sex, Apgar score, infant diagnoses including congenital malformations, and date of death.

7.2.2 Clinical Register of RA

The Early Rheumatoid Arthritis Register (ERAR)
This register was initiated 1994 as a surveillance register for early rheumatoid arthritis patients. The purpose was to evaluate the effect of new treatments in individual patients and to be able to study prescription patterns and outcome results in various parts of the country. At inclusion the NRN, date of onset of symptoms, date of diagnosis, ACR criteria and characteristics of the disease at onset, including RF status are collected. Data on treatment and clinical status are recorded at follow-up visits at 3, 6, 12, 18 and 24 months and therafter, whenever clinically relevant, or at least yearly [252]. The register is maintained and owned by the Swedish Society for Rheumatology. It is used in clinical practice and reporting to the register is done by the treating rheumatologist. All patients included in this register have given their informed consent to participate. The number of participating centres and their coverage has increased over time and the participating centres represent a mixture of small and large clinics or departments. All public and private rheumatology out-patient clinics participate, but the reporting can vary depending on factors such as availability of rheumatologists. At present, the register includes close to 10 000 patients.

7.2.3 Other

The Construction Workers Cohort
The Construction Industry's Organization for Working Environment, Safety and Health (Bygghälsan) provided out-patient services to construction workers all over Sweden between 1969 and 1993. Personal invitations to preventive health check-ups, sent out every second to third year, and advertisements at almost all major building sites in Sweden were used to reach both blue and white collar employees. Approximately 75% of the employees in the industry were participating but there is no information on whether non-attendants did not get an invitation or if they were unwilling to participate. The health check-ups took place at both stationary and mobile clinics staffed by nurses

and physicians. The total number of constructions workers who attended was 386000, who each made between one and 13 visits.
Between 1971 and 1975 exposure information on tobacco use was included in a self-administered questionnaire and double checked at the visit by attending staff. Another form, called the LS form, was completed by the staff. After the first quarter of 1975, the self-administered questionnaire was no longer in use, but the LS form remained. This meant that data collection about tobacco smoking was temporarily stopped. From mid 1978, an expanded LS form was introduced. On this form the attending nurses filled out detailed information about current and previous tobacco use including smokeless tobacco, Swedish moist snuff.

7.2.4 Conditions for register linkages

The Swedish national registers are regularly used by the register holders to produce statistics on deaths, health and disease, lifestyles etc. Researchers can get access to the registers through application to the register holders after approval from the ethics review board. The register linkages take place at the central agencies. There are different methods of protecting the integrity of the individuals in the registers. Most often the NRN of each person is stripped and replaced by a random number. This number is still unique to each person and can thus be used to match information from different registers. Sometimes a key to the NRNs is kept by the register holders enabling future linkages to add data not yet available at the time of the first linkage, but also giving a possibilty to perform validation of register information versus for example medical records. In some special situations the NRNs are not replaced by a random number, but may be delivered back to the researcher. The data is always handled according to special routines for data protection.

7.3 STUDY DESIGNS

7.3.1 Paper I

In this population-based case-control study, we investigated the importance of birth characteristics and early life infections on the risk of later RA and JIA.

We defined two groups of cases, RA and JIA, respectively. In the Swedish Hospital Discharge Register we identified all individuals, born 1973 or later, with a discharge diagnosis of RA at 16 years of age or above (n=208). Through linkage with the Early RAR we identified 143 partly overlapping cases born 1973 or later, the total number of RA cases being 333. RF status was determined as recorded in the ERAR and/or by the ICD code used in the Swedish Hospital Discharge Register. 153 (46%) RA cases were RF positive, 107 (32%) RF negative and 73 (22%) were not specified. As the RA cases were only 16 to 29 years of age at diagnosis, this study was limited to “early adult onset RA”. In the Swedish Hospital Discharge Register, we further identified 3,334 cases discharged with JIA. Of these, 230 had at some later stage also been discharged with an RA-diagnosis, but were analysed according to the first diagnosis made (JIA).

Through linkage with the Swedish Medical Birth Register we selected four same-sexed controls for each case, born at the same delivery unit the same year as the case and alive at the time of diagnosis of the case.

Exposure information was retrieved from the Swedish Medical Birth Register and the Swedish Hospital Discharge Register. The following variables were analysed: maternal age, maternal civil status, parity, feto-maternal blood group incompatibility, mode of delivery (vaginal *vs.* Caesarean section), singleton *vs.* multiple birth, date of birth, birth weight, gestational age, Apgar score at 5 minutes, malformations, neonatal and infant infections up to one year of age and maternal infections during pregnancy. Small for gestational age was defined as a birth weight for gestational age below -2 standard deviations (SD) according to the Swedish reference for fetal growth [34]. Appropriate for gestational age was defined as birth weight for gestational age from -2 to +2 SD. Large for gestational age was defined as birth weight for gestational age above +2 SD.

7.3.2 Paper II

In this matched case-control study, we investigated the role of infections during infancy, childhood and adolescence on the risk of developing RA later in life.

In the ERAR, we identified as cases all individuals diagnosed with RA 1995 through January 31st 2008, aged 16 or above. Since exposure information on infections 0-16 years of age in the Swedish Hospital Discharge Register was available from 1964 we excluded cases born before 1949 (who would not contribute any information on infections before 16 years of age), leaving a total number of 3038 RA cases.

For each RA case we randomly selected five controls from the Register of Total Population, matched to their case by year of birth, gender, marital status, and county of residence. Controls had to be alive at the time of the RA diagnosis of their corresponding case. In total, 15 187 controls were identified.

Exposure information on hospitalisations listing an infection at 0 through 15 years of age was retrieved from the Swedish Hospital Discharge Register using pre-defined discharge codes, ICD versions 7-10. Exposure to infection was further categorised according to age at infection ($\leq$ 1, 1-7, 8-15, and <16 years of age at infection), organ type of infection, and number of hospitalisations listing infection (0, 1, 2-3, >3). We also extracted information on diabetes mellitus and asthma, potential confounders possibly affecting the chance of being hospitalised for infections in childhood.

7.3.3 Paper III

In this population-based case-control study we assessed the risk of RA following vagotomy.

We identified two sets of cases. As a first set of cases we identified all individuals in the Swedish Hospital Discharge Register above 16 years of age who were discharged from inpatient care with RA at least once 1964-2001 and who had not at any point in time also been discharged with psoriatic arthritis, ankylosing spondylitis, or systemic

lupus erythematosus (n=63,092). As a second set of cases we identified all 2,458 individuals registered in the ERAR as of 2003.

Through linkage with the Register of Population and Population Changes we randomly selected two controls per case from the Swedish Hospital Discharge Register (n=125 404) and ten controls per case from the ERAR (n=24 357), matched to their case by year of birth, sex, civil status and region of residence. The controls had to be alive at the time of RA diagnosis of their cases.

Exposure information was retrieved from the Swedish Hospital Discharge Register. We identified all surgical procedures recorded from 1964 until one year before the date of diagnosis / first discharge with RA listing vagotomy or abdominal ventricular resection. To reduce the risk of reversed causality in the analyses based on cases identified in the Swedish Hospital Discharge Register and their controls, we only included surgical procedures occurring before the first hospitalisation listing any musculoskeletal diagnosis (e.g., unspecified mono- and poly-arthritis, arthralgias) rather than first hospitalisation specifically listing RA. Similarly, for cases identified in the ERAR and their controls, we only included exposures occurring at least one year before the registered date of diagnosis of RA. To assess the specificity of any observed association between vagotomy and RA, and to detect associations due to reversed causality (e.g., NSAID-related ulcers in patients with incipient or not yet hospitalised RA) or confounding e.g., smoke-related gastric ulcers [253], we identified related surgical procedures or medical diagnoses that normally do not include vagotomy (gastrorraphy, any gastric or duodenal ulcer). To detect operability-related biases, we also assessed the relative risk associated with a pre-RA cholecystectomy, for which there is no known association with RA [254].

7.3.4 Paper IV

In this cohort study we assessed and compared the risks of RA, ulcerative colitis, Crohn's disease, sarcoidosis and multiple sclerosis associated with smoking and use of Swedish moist snuff using the Construction Workers Cohort.

During the period 1975 to 1978 no information regarding tobacco use was collected, therefore this study includes subjects in the Construction Workers Cohort with at least one visit January 1 1978 or later, leaving a cohort of 300 637 subjects. Because of great individual differences in number and timing of repeated visits, probably partly driven by self selection, we only used exposure information from the first registered visit. Since only approximately five percent of the cohort members were women (n=14 982) and use of moist snuff among women is uncommon we restricted our analysis to men. We further excluded subjects with data irregularities and incomplete exposure information, so that the final cohort consisted of 277 777 subjects, figure 2.

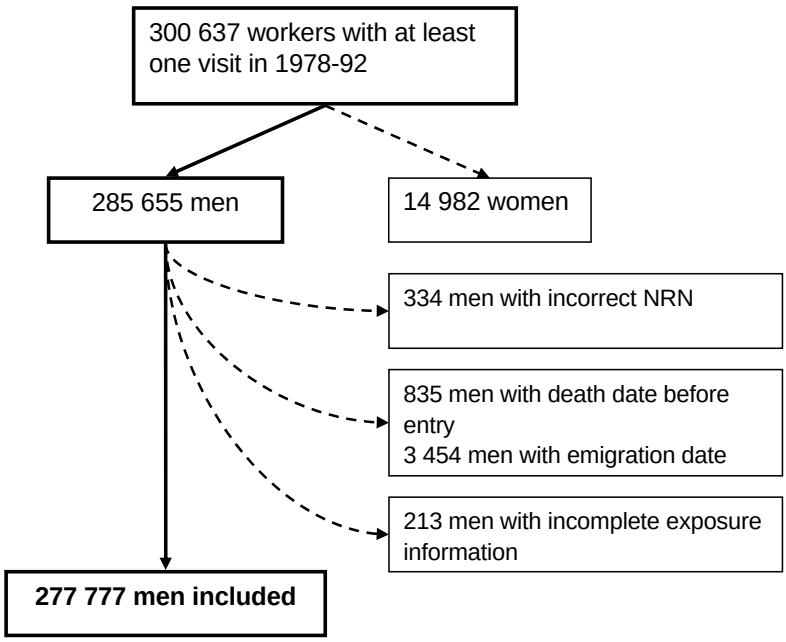

Figure 2 Schematic description of the cohort in study IV

Exposure information on tobacco use was categorised as follows: Use of Swedish moist snuff was categorised according to *user status* (never, former or current) and *amount* of moist snuff used per day (<22 grams or ≥22grams). Current use was defined as daily consumption of snuff. Ever snuff use was defined as current or former use. Smoking was categorised according to *user status* (never, former or current) and includes cigarette smoking, cigar smoking and pipe-smoking. Current smoker was defined as daily smoking. Ever smoker included current and former smokers.

Using the NRNs as linkage key the cohort was linked to the Swedish Hospital Discharge Register, Register of Population and Population Changes and Cause of Death Register. If an individual was living in a county with incomplete coverage of the Swedish Hospital Discharge Register, the entry date into the cohort was reset to the date of coverage. In each outcome analysis we also excluded subjects with the outcome disease already before the start of follow-up (RA n=12, UC n=138, CD n=47, sarcoidosis n=11 and MS n=45). In the Swedish Hospital Discharge Register we identified all hospital discharge files listing a main or contributory diagnosis of RA, UC, CD, sarcoidosis, and MS 1964 through 2004. Rheumatoid factor status was determined by the ICD code used for RA in the Swedish Hospital Discharge Register, although 51% of the RA cases had missing or incomplete data on RF status through this method. Censoring dates for death and emigration were obtained from the Causes of Death Register, and from the Register of Population and Population Changes, respectively. Each cohort member contributed person-years from the date of entry until

the date of any diagnosis (RA, UC, CD, sarcoidosis and MS), death, emigration or December 31, 2004, whichever came first.

7.4 STATISTICAL ANALYSIS

All analyses were conducted using SAS statistical software, (Cary, NC, USA)

Paper I
The association between the exposures (maternal, pregnancy and birth characteristics, infections during first year of life and maternal infections during pregnancy) and the risk of developing RA and JIA was estimated as univariate odds ratios with 95% confidence intervals (95% CI) using conditional logistic regression.

Although considering the tight correlation between factors such as birth weight, gestational length, and size for gestational age and the fact that these are rather to be regarded as markers of fetal growth than as actual biologic risk factors themselves, we chose to also perform multivariate analyses. In these models we included maternal age, civil status, season of birth, number of siblings, mode of delivery, gestational age and birth weight. For JIA, we also performed multivariate analysis with and without maternal smoking, but since maternal smoking was introduced in the Medical Birth Register first in 1983 it was not included in the multivariate model for RA.

Paper II
The association between infections and the risk of developing RA was estimated by calculating odds ratios with 95% CI using conditional logistic regression. Odd ratios were assessed overall, stratified by gender, RF status, and DAS28 during the first year with RA. We also performed analyses adjusted for childhood asthma and diabetes mellitus, potential confounders that may affect the chance of being hospitalised with infection before age 16.

Paper III
The association between the exposures (vagotomy/abdominal ventricular resection, gastrorraphy, gastric or duodenal ulcer, cholecystectomy) and the risk of RA was estimated as odds ratios including 95% CI using conditional logistic regression. Analyses stratified by time since vagotomy (1-4, 5-9, 10-19, ≥20 years) were also performed.

Paper IV
For each of the outcomes, incidence rates per 100 000 person-years standardised to the age distribution (five-year age categories) in the entire cohort were calculated. Relative risks for the association between tobacco use and the inflammatory diseases were estimated using Cox proportional hazards models taking age into account, with adjustment for region of residence.
Use of Swedish moist snuff and smoking are closely correlated. Therefore we performed two sets of models: First, ever smoking (yes/no) and ever snuff use (yes/no) were entered into a bi-variable model. Thereafter, we categorised each cohort member into either of the following mutually exclusive exposure categories of tobacco exposure: (i) ever smokers who were never users of moist snuff, (ii) ever users of moist

snuff who were never smokers, (iii) ever smokers who were also ever users of moist snuff, and (iv) never users of tobacco, i.e., never smokers who were never users of moist snuff. Separate models were subsequently fitted for each of these exposure categories (i) to (iii), all using non-tobacco users (iv) as reference. Models stratified by current and former, respectively, smokers were also fitted. Due to the very small numbers of former snuff users in each outcome group, analyses were only performed for ever snuff users.

The assumption of proportional hazards for smoking, snuff use and covariates was examined by the method of Shoenfeld´s partial residuals [255]. The results indicated that the proportional assumption was satisfied for all models.

8 RESULTS

8.1 PAPER I

Of the 333 RA cases 252 (76%) were female and 81 (24%) were males. Of the 3334 JIA cases 1800 (54%) were female and 1534 (46%) were males. The median age at diagnosis/first hospital discharge was 23 years of age for RA and 3 years of age for JIA.

Maternal, pregnancy and infant characteristics and risk of later RA
Low birth weight (less than 3000 g) and being small for gestational age were associated with a reduced risk of developing RA of borderline statistical significance (OR 0.7 95%CI 0.5-1.0 and OR 0.5, 95% CI 0.1-1.0 respectively). Preterm birth (≤258 days) was associated with a statistically non-significantly reduced risk (OR=0.6, 95%CI 0.4-1.1). By contrast, being large for gestational age and having three or more older siblings was associated with a non-significantly increased risk (OR 1.6, 95% CI 0.7-3.3 and 1.4, 95% CI 0.8-2.4 respectively). There was no association between the risk of RA and maternal age, maternal civil status, Caesarean section, multiple birth, Apgar score at 5 minutes, malformations, maternal-child blood group incompatibility or season of birth. Alternative categorisations (<2500 g, 2500-3999 g (reference), ≥4000g and <3000 g, 3000-4499 g (reference), ≥4500 g) only marginally altered our results. Multivariate analysis did not markedly affect the point estimates.

Maternal, pregnancy and infant characteristics and risk of later JIA
Being born after more than 42 gestational weeks (≥294 days) was associated with an increased risk of JIA of borderline statistical significance (OR=1.2, 95% CI 1.0-1.34). A borderline statistically significant reduced risk (OR= 0.7, 95% CI 0.5-1.0) was observed for individuals with an Apgar score at 5 minutes of 6 or less. Neither maternal age nor civil status, birth weight, small/large for gestational age, multiple birth, number of older siblings, malformations, maternal smoking, season of birth or maternal-child blood group incompatibility was associated with the risk of JIA. Multivariate analyses with and without maternal smoking hardly altered our results.

Infections and later risk of RA
Eighteen of the 333 RA cases and 51 of the 1392 controls were hospitalised for infection during the first year of life. Overall, hospitalisation for any infection during the first year of life was associated with a non-significantly increased risk of RA (OR=1.4, 95% CI 0.8-2.5). Analysis according to RF status revealed a stronger association for RF negative RA (OR=2.6, 95%CI 1.0-7.0) than for RF positive RA (OR=1.2, 95% CI 0.5-2.9), although both assessments were based on small numbers. In multivariate models which, besides infection, included maternal age, civil status, season of birth, number of siblings, mode of delivery, gestational age and birth weight, the associations with infection remained. Maternal infections during pregnancy were not associated with the risk of RA.

Infections and later risk of JIA
Four hundred eighteen of the 3334 cases and 954 of the 13 336 controls were hospitalised for infection during the first year of life. Being hospitalised for any

infection during the first year of life was associated with an increased risk of JIA (OR=1.9, 95% CI 1.7-2.1). Exploratory analyses of specific types of infections showed increased risks for respiratory (OR=2.0, 95% CI1.7-2.4), gastrointestinal (OR=2.3, 95% CI 1.8-2.8) and skin/soft tissue infections (OR=1.5, 95% CI 1.1-2.2). Multivariate analysis only marginally altered the point estimates. Maternal infections during pregnancy were not associated with the risk of JIA.

8.2 PAPER II

In this case control study, 196 (6.5%) of the 3038 RA cases and 752 (5.0%) of the 15187 population based matched controls were hospitalised with any infection at least once before the age of 16. The mean age at hospitalisation with any infection among cases and controls was 7 and 6 years, respectively and the median time from infection until inclusion in the ERAR was 23 years (range 2-40 years). Overall, hospitalisation for any infection before the age of 16 was associated with a moderately increased risk of RA (OR=1.4, 95% CI 1.1-1.6).

Age at infection
Analyses according to age at infection (categorised as during first year of life, 1 to 7 years of age and 8 to15 years of age, respectively) showed an increased risk of RA for infections at 8 to 15 years of age (OR=1.5, 95% CI 1.2-2.0). Being hospitalised for infection during first year of life was associated with a non-significantly increased risk (OR=1.3, 95% CI 0.8-1.9).

RF positive and RF negative RA
Analyses stratified according to RF status suggested no major overall difference between RF positive and RF negative RA with respect to hospitalisation for any infection before 16 years of age (OR RF positive RA = 1.4, 95% CI 1.2-1.8 vs. OR RF negative RA =1.3, 95% CI 0.9-1.8). When further stratified according to age at infection, hospitalisation for infections during the first year of life was associated with an increased risk, although not statistically significant, for RF negative (OR=1.8, 95% CI 0.9-3.9) but not for RF positive (OR=1.1, 95% CI 0.6-1.8) RA. By contrast, the association between infections at 8 to 15 years of age and increased risk of RA was largely confined to the RF positive subgroup (OR=1.8, 95% CI 1.3-2.3).

Number of infections
Analyses according to number of hospitalisations with infection suggested a trend of increasing risk of later RA with increasing number of hospitalisations with any infection (OR for one hospitalisation = 1.30, 95% CI 1.07-1.58, OR for two to three hospitalisations with infection = 1.47, 95% CI 1.03-2.10, and OR for four or more hospitalisations with infection = 2.63, 95% CI 1.18-5.86, p comparing 1 to > 3 infection = 0.09).

Organ type of infection
Borderline increased or statistically significant increased risks for RA were observed for respiratory tract infections at 1 to 7 years of age (OR=1.36, 95% CI 0.94-1.96), for gastrointestinal infections at 8 to15 years of age (OR=2.15, 95% CI 1.07-4.29), and for skin/soft tissue infections at 8 to15 years of age (OR=1.95, 95% CI 0.95-3.98).

Disease activity at RA diagnosis and during the first year following RA diagnosis
Median DAS28 at inclusion was 4.9 (range 1.1-8.1) for cases with a history of hospitalisation with any infection before 16 years of age and 5.1 (range 0.5-8.8) for cases without such a history (data available for 2 323 cases). By calculating the area under the curve of DAS28 measurments available during the first year of follow-up (data available for 1,752 cases), cases were categorized as high or low disease activity. When stratified by high or low disease activity similar odds ratios were noted in both groups.

Finally, adjustment for hospitalisation with asthma and diabetes mellitus before the age of 16 suggested little confounding by these factors (OR <5% changed).

8.3 PAPER III

Of the 63 092 RA cases identified in the Swedish Hospital Discharge Register 44 722 (71%) were female and 18 370 (29%) were male. Of the 2458 cases identified in the ERAR 1725 (70%) were female and 733 (30%) were male.

In all, 179 (0.28%) of the 63 092 RA cases identified in the Swedish Hospital Discharge Register and 304 (0.24%) of their matched controls had a history of vagotomy or abdominal ventricular resection (OR=1.2, 95% CI 1.0-1.4), with similar ORs for isolated vagotomy and for abdominal ventricular resection. Relative risks in the same range were observed for gastrorraphy (OR=1.8, 95% CI 1.3-2.6) and for gastroduodenal ulcer disease (OR=1.21, 95% CI 1.1-1.3) with little difference between gastric, duodenal or unspecified location. In contrast, a history of cholecystectomy was not associated with rheumatoid arthritis (OR=1.0, 95% CI 0.9-1.1).

Ten (0.41%) of the 2458 RA cases identified in the ERAR and 64 (0.26%) of their 24 357 matched controls had a history of vagotomy or abdominal ventricular resection (OR=1.6, 95% CI 0.6-3.8), but gastrorraphy (OR2.1, 95% CI 0.5-10) and gastroduodenal ulcer disease (1.3, 95% CI 0.9-1.8) were associated with increased risk in the same range. In contrast, a history of cholecystectomy was not associated with rheumatoid arthritis (OR=0.96, 95% CI 0.8-1.2).

8.4 PAPER IV

The final cohort consisted of 277 777 male construction workers who contributed with more than 5 million person-years of follow-up. The mean age at entry into the cohort was 36.

The relative risks for RA, UC, CD, sarcoidosis and MS in bi-variable models (comparing ever smokers and ever users of moist snuff to never tobacco users) are presented in table 2. Separate models of ever smokers who were never users of moist snuff, ever users of moist snuff who were never smokers and ever users of moist snuff and smoking in combination are presented in table 3 and 4. Separate models of current smokers who were never users of moist snuff and former smokers who were never users of moist snuff are presented in table 5 and 6. In all analyses, non-tobacco users comprised the reference.

Rheumatoid arthritis
During follow-up, a total of 797 cases of RA were identified, corresponding to an incidence rate of 14 per 100 000 person-years. Smokers had an increased risk of RA compared to non tobacco users and this risk was largely confined to RF positive RA. Current smokers had a higher risk for RA than former smokers. By contrast, users of moist snuff did not have an increased risk for RA compared to non tobacco users. Combined use of moist snuff and smoking was also associated with increased risk for RA.

Ulcerative colitis
During follow-up, 1014 cases of UC were identified, corresponding to an incidence rate of 18 per 100,000 person years. Ever smoking was associated with a slightly increased risk of UC compared to non tobacco users, but this was confined to former smokers. Ever use of moist snuff was not associated with risk of UC. Combined ever use of moist snuff and smoking was associated with an increased risk similar to that of former smokers.

Crohn's disease
During follow-up, a total of 628 cases of CD were identified, corresponding to an incidence rate of 11 per 100 000 person-years. Smokers had an increased risk of CD compared to non tobacco users. Current smokers had higher risk than former smokers. By contrast, use of moist snuff was not associated with the risk of CD. Combined ever use of moist snuff and smoking was associated with an increased risk of CD similar to that for smokers who did not use moist snuff.

Sarcoidosis
During follow-up, 342 cases of sarcoidosis were identified, corresponding to an incidence rate of 7 per 100,000 person-years. Smokers (ever, current and former) had a decreased risk of sarcoidosis compared to non tobacco users. By contrast, use of moist snuff was not associated with the risk of sarcoidosis. Combined use of moist snuff and smoking was associated with a decreased risk of sarcoidosis similar to that among current or former smokers who did not use moist snuff.

Multiple sclerosis
During follow-up, a total of 214 cases of MS were identified, corresponding to an incidence rate of 4 per 100,000 person years. Smokers had an increased risk of MS compared to non tobacco users. Current smokers had higher risk than former smokers. In bi-variable model ever use of moist snuff was not associated with MS. In restricted models, ever use of moist snuff (among never smokers) was associated with an increased risk of MS of borderline statistical significance. Combined use of moist snuff and smoking was associated with an increased risk of MS, which was somewhat lower than that among smokers who did not use moist snuff.

Table 2. Relative risks (RR) with 95% CI of rheumatoid arthritis, ulcerative colitis, Crohn's disease, sarcoidosis and multiple sclerosis among ever smokers and ever snuff users.

	Rheumatoid Arthritis	**Ulcerative Colitis**	**Crohn's Disease**	**Multiple Sclerosis**	**Sarcoidosis**
	RR(95%CI)	RR(95%CI)	RR(95%CI)	RR(95%CI)	RR(95%CI)
Never users of tobacco	1.0 reference n=129	1.0 Reference n=284	1.0 Reference n=157	1.0 Reference n=37	1.0 Reference n=145
Ever smoker	2.1 (1.7-2.5) n=641	1.3 (1.1-1.5) n=616	1.5 (1.2-1.8) n=405	1.9 (1.4-2.6) n=150	0.5 (0.4-0.5) n=135
Ever user of moist snuff	1.0 (0.9-1.2) n=168	1.1 (0.9-1.2) n=305	0.9 (0.8-1.1) n=174	1.0 (0.8-1.4) n=64	1.1 (0.9-1.4) n=103

Table 3. Relative risks (RR) with 95% CI of rheumatoid arthritis among ever smokers never using snuff, ever snuff users who were never smokers and among ever smokers and snuff users in combination.

	Rheumatoid arthritis	**RF positive**	**RF negative**
	RR(95%CI)	RR(95%CI)	RR(95%CI)
Never user of tobacco	ref n=129	ref n=40	ref n=34
Ever smoker, never use of moist snuff	2.3(1.9-2.7) n=500	2.2(1.6-3.2) n=162	1.1(0.7-1.7) n=67
Ever use of moist snuff, never smoker	1.2(0.8-1.8) n=27	1.2(0.6-2.7) n=8	1.3(0.6-2.8) n=8
Ever smoker and user of snus	2.0(1.6-2.6) n=141	2.3(1.5-3.5) n=49	1.2(0.7-2.0) n=21

Table 4. Relative risks (RR) with 95% CI of ulcerative colitis, Crohn's disease, sarcoidosis and multiple sclerosis among ever smokers never using snuff, ever snuff users who were never smokers and among ever smokers and snuff users in combination.

	Ulcerative colitis	**Crohn's disease**	**Sarcoidosis**	**Multiple sclerosis**
	RR(95%CI)	RR(95%CI)	RR(95%CI)	RR(95%CI)
Never user of tobacco	1.0 Reference n=284	1.0 Reference n=157	1.0 Reference n=145	1.0 Reference n=37
Ever smoker, never use of moist snuff	1.2(1.1-1.4) n=425	1.5(1.3-1.9) n=297	0.5(0.4-0.6) n=94	2.5(1.7-3.6) n=113
Ever use of moist snuff, never smoker	1.0(0.8-1.2) n=114	1.0(0.8-1.4) n=66	1.1(0.8-1.5) n=62	1.8(1.1-2.9) n=27
Ever smoker and ever user of moist snuff	1.4(1.1-1.6) n=191	1.4(1.1-1.8) n=108	0.5(0.4-0.8) n=41	1.9(1.2-3.1) n=37

Table 5. Relative risks (RR) with 95% CI of rheumatoid arthritis and sarcoidosis among current and former smokers who were never users of moist snuff.

	Rheumatoid Arthritis	**Sarcoidosis**
	RR(95%CI)	RR(95%CI)
Never user of tobacco	1.0 Reference n=129	1.0 Reference n=145
Current smoker	2.6(2.1-3.2) n=641	0.5(0.4-0.6) n=135
Former smoker	1.5(1.2-2.0) n=144	0.5(0.4-0.8) n=28

Table 6. Relative risks (RR) with 95% CI of ulcerative colitis, Crohn's disease and multiple sclerosis among current and former smokers who were never users of moist snuff.

	Ulcerative Colitis	**Crohn's Disease**	**Multiple Sclerosis**
	RR(95%CI)	RR(95%CI)	RR(95%CI)
Never user of tobacco	1.0 Reference n=284	1.0 Reference n=157	1.0 Reference n=37
Current smoker	1.1(1.0-1.4.) n=616	1.6(1.3-2.0) n=405	2.8(1.9-4.2) n=150
Former smoker	1.5(1.2-1.8) n=143	1.3(1.0-1.8) n=71	1.6(0.9-2.8) n=20

9 DISCUSSION

9.1 SETTING

The studies in this thesis are based on routinely collected register data covering the Swedish population, data from a semi-national clinical register and data from the Swedish Construction Workers Cohort, and the possibility to link data between these registers.

9.2 METHODOLOGICAL CONSIDERATIONS

9.2.1 Study design

Choosing the best, or most appropriate, study design in epidemiology is a delicate matter and often the result of a trade off between internal validity (that we measure what we aim to measure), external validity (to what extent the results can be applied to other populations) and efficiency (to get the most out of the time and money spent). For many research questions experimental study designs are not possible to perform due to ethical consideration (for example assigning a dangerous exposure to healthy individuals) and practical reasons. In those cases we have to rely on observational studies. In this thesis we used observational study designs.

A cohort study is a study where a group of individuals defined by a particular exposure at risk of an outcome is followed for the occurrence of the outcome. Cohort studies can be prospective or retrospective and they have many advantages. A retrospective cohort study can have prospectively recorded exposure information. Thus even in a retrospective cohort study, as long as exposure information is recorded in a prospective manner, exposure is recorded independently of the outcome and the risk of recall bias is minimised. This is the case for the cohort studies based on prospectively recorded data in Swedish national registers, even if exposure as well as the outcome dates several years back in time. In a cohort study many outcomes can be measured within the same cohort study, but, depending on design, several exposures may also be studied. Cohort studies are often well suited to study the effect of exposure changes over time and to study rare exposures. They can be used to calculate incidence rates or risks and their differences and ratios. Drawbacks of cohort studies are that they can be very expensive and time-consuming. In this regard, retrospective cohort studies using register based follow up may be very efficient. Large cohorts may be needed to obtain exposure information in order to measure the risk of disease and long follow up times may be needed when only a tiny minority of those who are at risk actually develop disease. There may also be problems with loss to follow up.

In a case-control study, study subjects are selected from the study base on the basis of having (cases) and not having (controls) developed the outcome. Cases and controls must be selected independently of the exposure status. Properly carried out, case-control studies provide information that mirrors what could be learnt from a cohort study, but are often more efficient, as the total number of included subjects can be kept lower. They are well suited to study uncommon diseases with unknown and possibly multiple causes and long induction time (where the exposure may precede the outcome by several decades). In the typical case, case-control studies can, however, only assess

estimates of the ratio of incidence rates or risks, not the incidences or risks per se. Like cohort studies, case-control studies can be both prospective and retrospective. When possible, prospectively recorded exposure information is preferred, both to ensure independence between exposure and case status and to minimise recall bias. The main threats to the validity of case-control studies lie in the difficulty of selecting cases and controls properly and in avoiding bias in the assessment of exposure.

In study I, II and III we used case-control designs. In all three of the studies exposure information was recorded prospectively. Study I is a nationwide case-control study, or more accurately two parallel exploratory case control studies. The outcome diseases JIA and RA are rare diseases, especially since RA was restricted to early onset RA, and several exposures were studied. A retrospective cohort study of all children born in Sweden between 1973 and 2002 would have been possible, but presented a less efficient alternative to a case-control design. Similarly, although studies II and III could theoretically have been designed as cohort studies, the case-control design used was more efficient.
In study IV, we used an existing cohort of male construction workers with prospectively recorded exposure information. This cohort was large enough to study rare diseases and it was also possible to readily study the outcome of five different inflammatory diseases. Although this is a retrospective study, the exposure information was recorded prospectively.

9.2.2 Internal validity

Internal validity means that we can trust the findings within the study population, i.e. that there is absence of systematic and random errors.
There are three major categories of systematic errors, or bias: selection bias, information bias and confounding.

Selection bias
The selection of subjects and factors that influence study participation may cause selection bias if the association between exposure and disease differs for those who participate and those who do not participate in the study or if exposure status influences the selection of cases and controls in a case-control study. In study I, II and III we selected cases and controls independently of exposure status. In study I and III we indentified RA cases in the Swedish Hospital Discharge Register, but also in the ERAR whereas all cases in study II were identified in the ERAR. The validity of the diagnosis of RA in the Swedish Hospital Discharge Register has been shown to be high. On the other hand, RA cases identified in the Swedish Hospital Discharge Register can not be claimed to be incident cases as no date of onset is recorded in this register. Using incident cases in a case control study is preferred since prevalent cases may be those with better survival/less co-morbidities, which in turn may be related to exposure status.
Over time, the health care system and the treatment of RA has changed. Historically, during the 1960s to 1980s a major portion of the RA cases were at some time hospitalised due to their RA diagnosis. This changed in the 1990s and today the major portion of RA cases are diagnosed and taken care of in daycare or outpatient clinics. Parallel to this development the ERAR was established which now gives us the ability

to identify incident cases. RA cases included in the ERAR fulfill the ACR 1987 criteria. Although all rheumatology clinics in Sweden are participating, the reporting varies so that we still do not have a nationwide coverage, but we do believe that the RA patients included in the ERAR are representative of patients taken care of in specialist rheumatology care. In study I and III we chose to use both the HDR and ERAR to identify cases. In study III we analysed them separately, but because of limited power due to few cases of early adult onset RA in study I we chose to analyse them as one set of cases.
In a case-control study, controls should be individuals who would have become cases had they developed the disease in question. The matched controls in study I were randomly selected from the study base which consisted of all individuals born in Sweden 1973 to 2002. In study II and III matched controls were randomly selected from the Swedish population. The HDR was not nationwide until 1987 and thus individuals who were living in a county without coverage before that could not become cases. Taking this into consideration, cases and controls were matched by county of residence and should therefore have the same chance of becoming a case.
In study IV, which was a cohort study, the cohort was selected on the basis of occupational status, but there was no selection within the cohort due to the exposure status. Only individuals working in the constructio industry could become cohort members. This means a selection of individuals who were able to work and may therefore be healthier than the general population ("the healthy worker effect") but also individuals who may be exposed to hazardous agents in their work and who may have different food, exercise, alcohol and tobacco using habits than the general population. As we did not compare this cohort directly with another and external cohort but rather performed internal comparisons, selection (or: restriction) will not affect the internal validity of the study, but may affect the external validity, which will be discussed later.

Information bias
Information bias can arise if information collected from or about the subjects is incorrect. The misclassification of information can be either differential or non-differential. Non-differential misclassification is a random misclassification that will dilute any observed association between exposure and disease. Differential misclassification arises when the misclassification of either the exposure in a subject is affected by the disease status, or if the misclassification of the disease status is affected by the subjects' exposure. It may affect the relative risk in any direction.
The exposure information in studies I-IV was recorded prospectively and thus there is no risk of recall bias and the exposure information was recorded independently of disease outcome. The quality of exposure information in the Medical Birth Register in study I is high, but information on maternal smoking was missing before 1983 [256]. Therefore analysis of maternal smoking was restricted to JIA. Exposure information on infections in study I and II, as well as exposure information in study III, was assessed in the Swedish Hospital Discharge Register. Although the quality of Swedish Hospital Discharge Register is high, a non-differential misclassification cannot be excluded. The collection of exposure information in study IV has been described above. By only using exposure data from the first health check-up (for reasons explained above), we cannot rule out the possibility that some individuals changed their tobacco using habits during follow-up. This could affect the results in our study and may be the explanation why we did not find a decreased risk of UC among current smokers. For the same reason, we

could not assess the effect of duration of tobacco use. Some studies have shown that snuff users are more prone to start smoking than non snuff users, whereas others have not [257, 258]. If snuff users are more prone to start smoking this could possibly affect the analysis of snuff users leading to results closer to those of smokers. In our study this could possibly have concealed a protecting effect of snuff use.
Not only exposure, but also case status in study I and III, and outcome in study IV was assessed through register based information. Although, as mentioned above, there is misclassification to some extent in the Swedish Hospital Discharge Register, the resulting bias is presumably more non differential than differential. With rheumatologist-based clinical RA diagnosis including information on the ACR criteria, missclassifiaction of the RA diagnosis in ERAR should be low.
Loss to follow-up is a potential bias in cohort studies. The coverage of the registers used for follow-up in study IV kept losses to follow-up to a minimum.

Reversed causality
Reversed causality arises if the exposure is an effect of the outcome. It can be a problem if the onset of disease actually occurs before the exposure. In diseases such as RA with an insidious onset, this may be a problem even when the cases in a case-control study are supposedly incident and in a cohort study although prevalent cases are excluded. In our studies of exposures mainly occurring many years before disease onset though, including incident cases is not as crucial as long as we do not measure exposure in close proximity of the RA diagnosis. In study III, where the exposure was vagotomy, this was a potential bias, why we took precautions to avoid and detect any such bias. We measured exposure occurring until one year before the diagnosis of RA/hospitalisation for RA. In the Swedish Hospital Discharge Register, we only measured exposures occurring before hospitalisation due to any musculoskeletal disorder, if present, instead of RA to minimise the risk of measuring exposure after the onset of an incipient RA. Use of NSAID and stress exposure accompanying an incipient RA may lead to gastric ulcers. We therefore identified related surgical procedures or medical diagnoses that normally do not include vagotomy (gastrorraphy, any gastric or duodenal ulcer) and found even stronger associations than those observed for surgical procedures including vagotomy. This indicates a possible reversed causality, but may also be a result of confounding due to smoking, which is a risk factor both for gastric ulcer and RA.

Confounding
Confounding can be explained as mixing/confusion of effects. The definition of a confounder, or a confounding factor, is a factor that is associated with both the disease and the exposure but not an effect of the exposure. Thus factors in the intermediate steps in the pathway from exposure to disease are not confounders (at least not for this reason, although they might be confounders thruogh other properties). As soon as potential confounders are unevenly distributed among the exposed and non exposed groups or among cases and controls, the relative risks may be affected in either direction. Confounding can be dealt with using three principle approaches: restriction, matching and statistical adjustments.
To be able to control for confounders the confounders need to be known. In study I to III cases and controls were matched on age, gender, county of living (in study I delivery unit) and civil status (study II and III). In these studies we did not have

information on other indicators of social class than civil status and could therefore not match or adjust the analysis for this potential confounder in other ways. Exposure to infections in study I and II could be a marker for some factors associated with poorer socioeconomic background, which in itself carries no biological properties. We also lacked information about tobacco using habits and other potential confounders such as occupational exposure, use of oral contraceptives and obesity. The results in study III are indeed compatible with a confounding by smoking. In study II, analyses adjusted for childhood asthma and diabetes mellitus, both of which are potential confounders affecting the risk of being hospitalised, did not affect the relative risks associated with infections. Multivariate regression analyses in study I did not markedly alter the point estimates.

Analyses in study IV were restricted to men and adjusted for region of recidence and age. In preliminary analyses, adjusting for body mass index had no impact on the risk estimates and was not kept in the final analyses. Smoking and use of moist snuff are highly correlated why we both performed bi-variate analyses of ever smokers and ever users of moist snuff and analyses restricted to smokers who were never snuff users and snuff users who were never smokers to avoid confounding. For MS, ever use of moist snuff in the bivariate model was not associated with disease risk, whereas ever use of moist snuff among never smokers was associated with an increased risk of borderline significance. The explanation for this finding is unknown, but may be explained by a chance finding, varying pattern of use of moist snuff in smokers and non-smokers or a true biologic effect of combined use. Since all analyses were performed within the cohort of construction workers, confounding by socio-economic factors may be limited. Alcohol consumption has been associated with a somewhat reduced risk of RA. Several studies have indicated an association between alcohol consumption and use of snuff, but also with smoking [259]. A protecting effect would then be seen in both exposure groups, which seems unlikly with regards to the present results.

In study III, we searched to assess any operability related bias, i.e. the possibility that cases would not have the same chance to have been operated on as controls. Therefore, we included cholecystectomy, not known or presumed to be associated with RA risk, as an exposure in the analysis, but found relative risks close to unity.

Chance

Observed associations may be due to chance. Statistical tests of significance or confidence intervals can help judgement of whether a given result is more or less probable to be a chance finding. If sample size and power is insufficient even a null results may be due to chance. As important though, is to evaluate the finding with respect to the a priori hypothesis, biological plausibilty, strength and specificity and temporality of exposure and outcome.

In study I, the number of RA cases was relatively small, which led to limited power in the analyses of RF-positive and RF-negative subsets. The number of controls for the RA cases was 10 per case. Usually, increasing the case-control ratio 5 marginally affects the precision, but when having rare exposures and the cost of adding controls is negligible, it is possible to minimise the risk of controls affecting the precision by using a higher ratio. One of the strengths in study III was the large study size which enabled us to study the rare exposure of vagotomy. The large size of the cohort in study IV, the

relative high exposure prevalence and the long and complete follow-up contributed to a high power.
The probability of a chance finding increases with the number of statistical tests performed. In exploratory studies such as study I and II, this has to be considered. The relative consistency in the findings of size at (low birth weight and small for gestational age) and timing of birth (preterm) and reduced risk of RA suggests that the observed results may be a true association. Because of the biological plausibilty, strength and precision of the association between infections and the risk of JIA and RA, chance is an unlikely explanation for this finding.

9.2.3 External validity

Study I and III were population based studies, thus permitting application of the results to all individuals in the catchment area, i.e. the whole of Sweden. By definition, the cases identified in the Swedish Hospital Discharge Register are hospitalised and may therefore represent a selection of more severe cases. However, the proportion of RF-positive and RF-negative RA cases identified in the Swedish Hospital Discharge Register is similar to what is found in other studies, and, as previously stated, hospitalisation tresholds for RA have traditionally been low. The ERAR used to identify cases in study II has a catchment area that encompasses a major part of Sweden and represents a mixture of small and large rheumatology clinics.
The observed incidences of RA (and the other outcomes) in study IV are also in broad accordance with those previously reported for males in the age-groups under study [16, 118, 249, 260-265]. On the other hand, other exposures in this cohort (such as other inhaled agents) may put a limit to the generalisability of our results in this study, as does the fact that all subjects were males. The RA cases in study I were limited to early adult onset RA, thus our study results may not be true for later onset RA. This was one of the reasons for performing study II, which indicated similar associations for infections under 1 year of age and RA. Furthermore, JIA comprises a heterogenous group of patients rendering difficulties to generalise the results to any specific subtype of JIA. It should also be pointed out that exposure to infection in study I and II was measured as being hospitalised for infections, thus only measuring exposure to more severe infections. The results should therefore not be interpreted to hold for being exposed to any infection. In fact, the observed associations might equally well reflect severity of infection as the risk of acquiring infection.

9.3 FINDINGS AND IMPLICATIONS

9.3.1 Early life factors and later risk of RA or JIA

The finding of a modestly reduced risk of early adult onset RA associated with factors related to fetal growth restriction and short gestational age has not been found in earlier studies and was not confirmed in a recently published study [145-147]. Conversely, our results do not support earlier findings of an association between high birth weight and the risk of developing RA [145, 146]. On the other hand, the suggested protection of having been breastfed in the study by Jacobson et al. was not confirmed in a recently published study from the Nurses Health Cohort [145, 147]. These inconsistent findings elucidate the difficulties of performing easily comparable epidemiological studies. There are several tentative explanations for these inconsistencies. The four studies span

over different birth cohorts from 1921 to 1986, which might reflect a change over time of the role of factors related to size at and timing of birth, which themselves may have varied by birth cohort. Our study was limited to early adult onset of RA (median age at diagnosis 23), whereas the median age at diagnosis in the other studies was 45, 46 and 58. The study by Jacobson et al. was hampered by small sample size and limited statistical precision. Additionally, exposure data was prospectively collected by midwifes in our study and in the study by Jacobson et al., both performed in Sweden whereas data on perinatal characteristics in the two studies of the Nurses Health Cohort Study was selfreported from US nurses.
Our results did not indicate any association between size at birth and JIA. Only one earlier study has investigated perinatal riskfactors in the context of JIA [106]. In this study by Jaakkola et al, investigating the role of maternal smoking during pregnancy and the risk of chronic arthritis during the first seven years of life, non-significantly increased odd ratios were observed both for low and for high birthweight. This study though, was based on only a total of 75 cases and is an example of the difficulties performing epidemiological studies due to the ambigous terminology of JIA. Fourtyfour of the cases in that study were classified as adult RA although being under seven years of age and only 31 as juvenile arthritis. We noted a somewhat increased risk of JIA when born at gestational age of 294 days or greater, which may be a chance finding and needs to be confirmed in other studies.

9.3.2 Infections during infance, childhood and adolescence and later risk of RA / JIA

The overall modest non-significant association between being hospitalised for infection during first year of life and risk of early adult onset RA noted in study I was more pronounced in the RF-negative subset than in the RF-positive subset. In study II, we found an increased risk, though not statistically significant, for RF negative RA, but no risk for RF positive RA. This was well in line with our previous results. The somewhat lower point estimate in study II may be partly explained by the fact that information on neonatal infections was included in study I and not in study II, but may also be due to the fact that study I was limited to early adult onset RA. The median age at diagnosis in study I was 23 as compared to 41 in study II.
With respect to childhood infections occurring after the first year of life in study II, the most pronounced risk was observed for infections, of several types, occuring between 8 and 15 years of age. In contrast to infections during the first year of life, this increased risk seemed stronger for RF positive than RF negative RA. We largely lacked information on infectious agent but instead based the exploratory analyses of type of infection on organ-related diagnoses of infections. The heterogeneous pattern of different subtypes of infection should be interpreted with caution due to the exploratory nature of the study, but at least suggests that individuals who will later develop RA have a different pattern of childhood infections than the general population.
The role of infections during childhood/adolescence and later risk of developing RA has, to our knowledge, not been assessed in other studies, but in a study of RF positivity in adult life (irrespective of RA diagnosis) by Edwards et al., a reduced risk for being RF positive was observed among women sharing bedroom during childhood, which was used as a proxy for infectious exposure [148]. On the other hand, when measuring clinical infections during first year of life, an association with increased risk

of anti-nuclear- and anti-cardiolipin antibodies in adult life was indicated [266, 267]. Measuring proxies of an exposure may measure the effect of other exposure(s) than the one aimed to study. This has been well illustrated in the context of atopy/allergy. A Danish study by Benn et al. showed that clinically apparent infections occurring early in life was associated with an increased risk of atopic dermatitis while the inverse was true for classically used proxies of infectious exposure indicating exposure to microbes rather than clinically overt infections [268].

In study II, we note with interest that the number of hospitalisations with infection was associated with later risk of RA, suggesting a cumulative effect of multiple exposures similar to what has also been found in the studies of early life infections and atopic dermatitis [268].

Rather than a sign of a causal, although not necessarily direct, link between childhood infections and risk of RA our findings may reflect an increased occurrence of childhood infections as the result of shared susceptibility, i.e., that the same genetic set-up increases the risk for both childhood infections and RA, or that an altered or enhanced immune responsiveness may lead to a more serious course of infectious disease resulting in hospitalisation. Measuring infections as being hospitalised for infection, precluds a full distinction between any effects of infections per se and by their severity. In animal models of adjuvant arthritis caused by injection of heat-killed Mycobacterium tuberculosis, multiple factors (both genetic and environmental) contribute to susceptibilty/resistence of different rat strains to adjuvant arthritis [269]. For example certain rat strains raised in a germ-free environment are susceptible to adjuvant arthritis, whereas rats raised in a conventional environment acquire resistence to adjuvant arthritis. Recently, it has been demonstrated that the fetal immune system can generate suppressive T regulatory cells (Tregs), by which the fetus can establish tolerance to foreign and self antigen present already during development in utero [270]. Interestingly, a new model for "the hygiene hypothesis" has been described, suggesting that exposure to harmless microorganisms and helminth infections leads to an activation of Tregs down regulating autoimmunity/inflammation [271]. On the other hand, legitimate danger signals, such as those of pathogenic infectious agents, interfere with this balance and cause an aggressive immune response required to clear the invader [271]. Treg cells have been suggested to be involved in the pathogenesis of autoimmune disease and a deficit in and functional impairment of Treg cells have been shown in early RA patients [272, 273]. Thus the fine tuning of the immune system through Treg cells and Teffector cells might, in susceptible individuals, be affected by pathogenic infections or by the severity of infection/infection load. In this regard, it should be pointed out that our findings do not support the role of a single infectious agent in the pathogenesis of RA, but rather an unspecific effect of exposure to any infection or infections.

The finding of an almost doubled risk for JIA following hospitalisation for infection during the first year of life in study I, is striking. The notion that an infection triggers onset of chronic arthritis in children has been investigated in several studies but is yet to be proved [101-103, 105]. Studies so far have focused on finding evidence for infection in patients with established disease but no studies have so far investigated early life infections and risk of later JIA development. As the median age of diagnosis of JIA was low (3 years of age) we cannot exclude that one explanation for the observed association is the occurrence of some form of reactive arthritis or infectious arthritis in the very youngest cases, although post-infectious arthritis is usually most frequent in

older children [274]. The main limitation of our study is the fact that the ICD classification used to identify JIA during the study period did not allow further subtype categorisation. In spite of this, our results shed new light on the etiology of JIA and calls for confirmation and more detailed assessments.
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9.3.3 The inflammatory reflex, tobacco and inflammatory diseases

In study III, the finding of a slightly, non-significantly, increased risk for RA following a surgical procedure involving vagotomy was not specific for vagotomy, but observed also for gastric ulcer disease and surgical treatment of gastric ulcer not including vagotomy. Since smoking is a risk factor for peptic ulcer and rheumatoid arthritis, our results might thus be due to confounding by smoking [275]. Furthermore, as stated above, we took precautions to avoid reversed causality, but cannot exclude that such bias may partly explain the observed increased risks.
Although quite impressive results have come out of experimental studies of vagotomy and vagus stimulation in animal models of acute inflammation, the effects of the inflammtory reflex on chronic inflammation remain unstudied. Vagotomy in our study was truncal or selective, isolated or as part of a surgical resection of the abdominal ventricle including its vagal branches. When we performed our study little was known about the relative contributions of the vagus nerve branches in the cholinergic anti-inflammatory pathway. As mentioned in chapter 2.5.2, recent studies published after our study was performed, indicate that the spleen is an important source of TNF production that is regulated by the anti-inflammatory effects of vagus nerve stimulation. It has further been shown in animal models that intact vagal innervation via the common celiac branches is required for vagus nerve regulation of the splenic and systemic TNF and that ventral subdiaphragmatic vagotomy is not sufficient to inhibit the effects of cervical vagus nerve stimulation [180]. Thus, vagotomy, as defined in our study, may not have been sufficiently extensive to inhibit the potential effects of the cholinergic anti-inflammatory pathway.
The results in study IV confirm and extend previous observations from various study designs that smoking is a risk factor for (seropositive) RA, CD, and MS, that quitting smoking reduces these increased risks, that smoking cessation is a risk factor for ulcerative colitis, and that smoking reduces the risk of sarcoidosis. In sharp contrast, no evidence of any significant association was noted between use of moist snuff and the risk for any of these chronic inflammatory diseases, with the exception of a borderline increased risk for MS with snuff use among never-smokers.
Despite the potentially strong effects of nicotine on the human immune system described in chapter 5.1.2, our findings suggest that the role of cigarette smoking in RA, CD, UC, sarcoidosis and MS is mediated through other mechanisms than through nicotine. With respect to RA, the role of smoking might be parallel to that of other inhaled non-nicotine containing irritants such as silica, possibly mediated through initiation of citrullination of certain proteins in the lungs as suggested by Klareskog et al [74]. In the light of previous finding of a possible beneficial effect of transdermal nicotine in active UC [203] the lack of protective effect of use of moist snuff for UC in our study is somewhat surprising. On the other hand, one earlier case-control study on smoking and use of moist snuff as risk factors for UC and CD did not show any association between use of moist snuff among non-smokers and risk of UC or CD, but

increased risks associated with snuff use among ever smokers [243]. These results though were based only on 15 and 11 exposed cases respectively. With regards to sarcoidosis, use of moist snuff did not seem to influence the risk of sarcoidosis. Our results would thus argue against nicotine as an important protective agent against sarcoidosis, but indicate other constituents in cigarette smoke as responsible for the protective effect.

The absence of an effect by nicotine and Vagotomy (although the shortcomings of our study discussed above), and thus indirectly of the inflammatory reflex, in the context of chronic inflammation may have several explanations. Studies of nicotine addiction show that chronic exposure to nicotine causes both desensitization and upregulation of α7nAChR in the brain [276]. Chronic exposure to nicotine may thus cause habituation of the neural/immune responses. There is also accumulating evidence indicating that innervation of lymphoid organs is not a static phenomenon. Animal models of adjuvant and collagen-induced arthritis have shown changes in number and sprouting of splenic nerve endings accompanied by changes in spleen cytokine release [277, 278]. A neural induced-desensitisation (or sensitisation) of macrophages has also been hypothesised [177]. Further, in a study of experimental arthritis, α7 agonist suppressed arthritis, whereas the exacerbation of collagen induced arthritis in vagotomised animals did not reach statistical significance [186]. Although the results in our studies do not support a major effects of the inflammatory reflex in chronic inflammatory diseases, it is interesting to note that research of innovative therapies with α7 agonists and vagus nerve stimulation is ongoing [279].

10 CONCLUSIONS

- Factors related to fetal growth and timing of birth may be important in the etiology of RA
- Infections during childhood may be of etiological importance in the pathogenesis of RA
- Infections during the first year of life, but not other perinatal characteristics, may be of importance for the risk of developing JIA
- Surgical vagotomy as performed in humans has no effect on the risk of developing RA
- Smoking is a strong risk factor for chronic inflammatory diseases
- Use of moist snuff is not a risk factor for RA, UC, CD and sarcoidosis
- Exposure to (inhaled) non-nicotinic components of tobacco smoke seem to be more important than nicotine itself in the etiology of chronic inflammatory diseases
- A major effect of the inflammatory reflex in the *etiology* of chronic inflammatory diseases seems less likely

11 FUTURE RESEARCH

Our results on childhood infections and later risk of developing RA and JIA were based on hospitalisation for infection raising the questions of whether there is an association also with milder infections not leading to hospitalisation, or an association with the accumulating effect of recurrent infections. Is it the effect of a severe infection leading to a pronounced immune response that is of importance, or is the clinical infection a marker of (in this regard) a deficient immune response? Are infections closer to disease onset also of importance? What are the mechanisms behind this seemingly unspecific association between infections and risk of RA and JIA? Are there differences according to SE status or other genetic markers of RA risk?

The results in the study on perinatal risk factors and risk of later RA were based on a limited number of cases and controls and were assessed only in relation to early adult onset RA. A larger study, also including later onset RA, is needed to confirm these findings, and, if substantiated, to further explore the pathway between these exposures and the development of chronic inflammation.

With respect to snuff use, further studies examining the effects of smokeless tobacco on the risk of developing chronic inflammatory diseases are needed to confirm our findings. This can be evaluated in the context of, for example, the EIRA project. Although substantial work has been done to sort out the effects of smoking on the immune system in humans, our results underscore the complexity of smoking effects and need for further mechanistic studies of smoking, in particular with regards to chronic inflammartory diseases.

Although we could not observe an effect of exposure to nicotine, administered as a content of moist snuff, on the risk of *developing* RA, UC, CD and sarcoidosis, pertinent questions include whether there are any effects of use of moist snuff on the *course* of those diseases? Smoking RA patients have a more severe course of disease, but what about RA patients using moist snuff? What happens if RA patients who smoke follow their doctor's advice and quit smoking, but starts using snuff or nicotine replacement? Indeed, although our results so far offer little support for the hypothesis that the inflammatory reflex would impact the development of chronic inflammation, pertinent future questions include its effect on the modulation of inflammation among humans with established inflammatory disease.

12 SVENSK SAMMANFATTNING

Reumatoid artrit (RA) är en kronisk systemisk inflammatorisk ledsjukdom som orsakas av en kombination av kända och okända genetiska och miljömässiga faktorer. Den här avhandlingen syftar till att öka kunskapen om varför ledgångsreumatism uppkommer, specifikt till att undersöka betydelsen av den "inflammatoriska reflexen", infektioner under barndomen och tidiga händelser i livet (perinatala faktorer) för risken att utveckla RA. Arbetet baseras på fyra epidemiologiska studier.

I den första studien undersökte vi betydelsen av perinatala faktorer och infektioner under första levnadsåret för risken att senare utveckla juvenil idiopatisk artrit (barnreumatism) och RA hos unga vuxna. Vi gjorde en fall-kontroll studie där vi använde data från slutenvårdsregistret, svenska tidiga reumatoid artrit registret och medicinska födelseregistret. Risken för barnreumatism och reumatoid faktor (RF) negativ RA var ökad hos de som vårdats på sjukhus för en infektion under första levnadsåret. En något minskad risk för RA sågs hos de som hade låg födelsevikt, var små för tiden och var förtidigt födda.

I den andra studien ville vi, i en större studiepopulation, verfiera fynden av en ökad risk för RA efter infektion tidigt i livet och även studera effekten av infektioner under hela uppväxten. Med hjälp av data från tidiga reumatoid artrit registret och slutenvårdregistret gjorde vi en fall-kontroll studie och fann att de som hade sjukhusvårdats för en infektion före 16 års ålder hade ökad risk att utveckla RA senare i livet. De som hade sjukhusvårdats för en infektion före ett års ålder verkade ha en ökad risk för RF negativ RA, medan de som hade sjukhusvårdats för en infektion mellan 8 och 15 år hade en ökad risk för RF positiv RA.

I den tredje studien ville vi undersöka betydelsen av den inflammatoriska reflexen för risken att utveckla RA. Vi gjorde en fall-kontroll studie där vi studerade om de som genomgått en operation där man tog bort en del av vagusnerven hade en ökad risk för att utveckla RA. Resultaten kunde inte visa att de som hade genomgått operation av vagusnerven hade en ökad risk för RA.

I den fjärde studien var syftet att indirekt studera betydelsen av den inflammatoriska reflexen genom att undersöka hur nikotin påverkar risken att utveckla RA och andra kroniska inflammatoriska sjukdomar. Genom att jämföra risken för RA, ulcerös kolit, Crohn's sjukdom, sarkoidos och multipel skleros hos de som snusar med de som röker ville vi urskilja betydelsen av nikotin från betydelsen av andra beståndsdelar i tobaksrök. Detta gjorde vi i en stor kohort studie av män som deltagit i hälsokontroller inom byggindustrin i Sverige. Resultaten beträffande rökning och risken att utveckla dessa sjukdomar stämde väl överens med tidigare observationer, nämligen att rökning ökar risken för RA, Crohn's sjukdom och multipel skleros, att rökstopp minskar dessa risken, att före detta rökare har ökad risk för ulcerös kolit och att rökning minskar risken för sarkoidos. Däremot fann vi ingen ökad risk för dessa sjukdomar hos de som snusar förutom möjligen en liten ökad risk för multipel skleros. Därmed fann vi inget övertygande stöd för hypotesen att den inflammatoriska reflexen påverkar risken att utveckla kroniska inflammatoriska sjukdomar.

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