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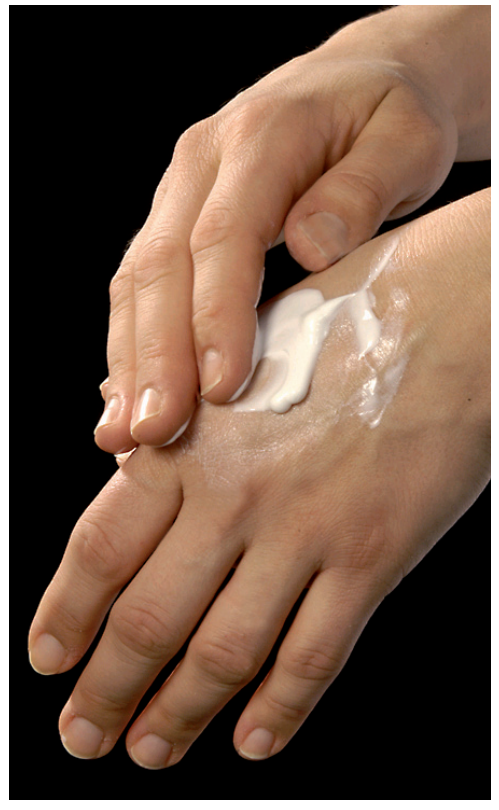

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ASPECTS OF THE ROLE OF MINERAL OIL AS IMMUNOLOGICAL ADJUVANT IN RHEUMATOID ARTHRITIS

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Carl Milles The hand of God. © Carl Milles / BUS 2007 Photograph Allan Larsson

To my patients
with thanks
and the hope of being some help

ABSTRACT

Environmental factors are likely contributing factors in the development of Rheumatoid Arthritis (RA), an autoimmune disease, for which etiologic factors are still poorly known. Mineral oils are efficient adjuvants which increase immunological responses and are used in animal immunization and for induction of different experimental autoimmune diseases including arthritis.

The relationship between environmental exposure to mineral oil and the risk of developing RA is the subject of this thesis. Both animal experimental studies and human epidemiological studies were performed in order to investigate the possible role of mineral oils in the development of arthritis. A special emphasis was devoted to investigations of whether exposure to cosmetics containing mineral oils was associated with arthritis in a similar way as exposure to the mineral oils themselves.

Experimental studies using the Dark Agouti rat (DA rat) showed that five of the eight tested common, commercial cosmetic products containing mineral oil induced arthritis in DA rats after immunisation in the skin. One cosmetic product, so called baby oil, also induced a mild transient arthritis after percutaneous exposure.

Two types of epidemiological studies were performed to investigate the relationship between occupational exposure to mineral oil and the risk of developing RA. First, a register-based cohort study was used to compare the cumulative incidence of RA between individuals with different occupations. The study population comprised of subjects who in 1980 lived in one of 13 Swedish counties and were born 1905-1945, and stated the same occupation in the census of 1960 and 1970. The study population was followed concerning hospital care for RA in 1981-1983 by linkage to the Swedish Hospital Discharge Register. A small increased risk of RA was observed in farmers, upholsterers, lacquerers, concrete workers and hair-dressers and in several occupations such as toolmakers, machinery and engine repairmen. Many of these occupations are associated with exposure to organic solvents and mineral oil, and this register study triggered our interest in initiating a case-control study investigating the environmental risk factors for RA, including the risk confined by mineral oil exposure. The case-control study comprised 1419 incident RA cases and 1674 controls. Men occupationally exposed to mineral oil were observed to have an increased risk of developing RA (Relative risk (RR) = 1.3, 95% confidence interval (CI) = 1.0-1.7). Mineral oil exposure was associated with a particularly high risk of developing rheumatoid factor positive RA (RR =1.4, 95% CI =1.0-2.0) and anti-citrulline positive RA (anti-CCP^{+}RA) (RR =1.6, 95% CI=1.1-2.2). The highest risk of developing anti- CCP^{+}RA (RR=1.7, 95% CI=1.1-2.6) was observed after exposure to hydraulic oil. The number of women occupationally exposed to mineral oils was too few to allow meaningful analyses.

Association between cosmetic usage and the risk of developing RA was also investigated in the case-control study. However, no increased risk through use of common skin care products such as body lotions and skin creams was observed.

Conclusion

This thesis demonstrates that environmental exposure to mineral oil by itself, as well as being a component of cosmetics, can induce experimental arthritis in rats with certain genetic characteristics, and that occupational exposure to mineral oils is associated with an increased risk of developing RA. Our epidemiological study gave no evidence that use of cosmetics containing mineral oils was associated with an increased risk for RA.

Keywords: Rheumatoid arthritis; adjuvant; mineral oil; experimental; DA rat; epidemiologic; cohort; case-control.

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LIST OF PUBLICATIONS INCLUDED IN THE THESIS

This thesis is based on the following publications and manuscripts, which will be referred in the text by their Roman numerals.

I. Sverdrup B, Klareskog L, Kleinau S. Common commercial cosmetic products induce arthritis in the DA rat. Environmental Health Perspectives 1998;106:27-32.

II. Lundberg I, Alfredsson L, Plato N, Sverdrup B, Klareskog L, and Kleinau S. Occupation, occupational exposure to chemicals and rheumatological disease. A register based cohort study. Scand J Rheumatol 1994;23:305-10

III. Sverdrup B, Kallberg H, Bengtsson C, Lundberg I, Padyukov L, Alfredsson L, Klareskog L; Epidemiological Investigation of Rheumatoid Arthritis Study Group. Association between occupational exposure to mineral oil and rheumatoid arthritis: results from the Swedish EIRA case-control study. Arthritis Res Ther. 2005;7(6):R1296-303. Epub 2005 Sep 23.

IV. Sverdrup B, Kallberg H, Klareskog L; Alfredsson L, Epidemiological Investigation of Rheumatoid Arthritis Study Group. Usage of skin care products and rheumatoid arthritis. Submitted.

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ANA	Antinuclear antibodies
Anti-CCP	Antibodies to citrulline-containing peptides
Anti-CCP^{+} RA	Anticitrulline positive rheumatoid arthritis
Anti-CCP^{-} RA	Anticitrulline negative rheumatoid arthritis
APC	Antigen presenting cells
B cell	Lymphocyte derived from the bone marrow
BCG	Bacillus Calmette-Guerin
CD	Cluster of differentiatian
CI	Confidence interval
DA	Dark Agouti
EIRA	Epidemiological investigation of rheumatoid artrhitis
ELISA	Enzyme-linked immunosorbent assay
FCA	Freund's complete adjuvant
FIA	Freund's incomplete adjuvant
HLA	Human leucocyte antigen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
JEM	Job exposure matrix
LC	Langerhans cell
LPS	Lipopolysackaride
MHC	Major histocompatibility
MMP	Matrix metalloproteinase
NK	Natural killer
Neg	Negative
PAMPS	Pathogen-associated molecular patterns
Pos	Positive
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RF^{+} RA	Seropositive rheumatoid arthritis
RF^{-} RA	Seronegative rheumatoid arthritis
RR	Relative risk
SAS	Statistical analysis system
SE	Shared epitope
SLE	Systemic lupus erytematosus
T cell	Thymus-derived lymphocyte
TCR	T cell receptor
TGF	Transforming growth factor
Th	T helper
TNF	Tumor necrosis factor

1 FOREWORD

My father, my grandfather and grandfather's father were all craftsmen and artisans, thus I looked upon the hand as the most important part of the human body. The deformed aching hand of rheumatoid arthritis (RA) that met me at the rheumatologic department had grabbed hold of me, since as a young physician I started working as rheumatologist.

In old China, a doctor who could not cure half of his patients was a bad doctor. I could not.

I started to read. But my book could not help me. RA patients were described as asthenic, old women. A psychologist declared that the joints were deformed owing to inhibited anger.

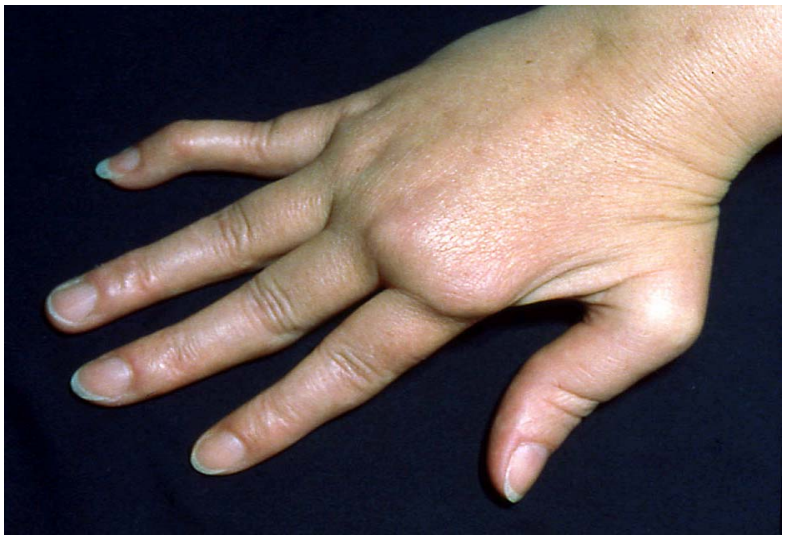
However, my impression was that RA patients were rather very strong and hard working people.

The old Greek doctor Hippocrates said that every patient carried the source of his disease with him.

So I tried to see, open my entire mind, my eyes, ears, and other senses. The patients that I had met at the department of medicine and emergency often were dirty, with a smell of perspiration and alcohol; the patients at the department of rheumatology were the opposite. Even if they could hardly move their arms or hands they were clean, sober with ironed blouses and well-undulated hair.

I read about RA epidemiology and experimental arthritis and there were many things I could not understand, but I noticed that little word oil, often occurring in the "Materials and methods".

With an adjuvant and an antigen you will have an autoimmune response. But, maybe it was *the adjuvant!* that was of interest and not the different antigens that had been surrounding us since the beginning of mankind.



2 INTRODUCTION

2.1 THE IMMUNE SYSTEM

Our defence against infectious agents, invading parasites, bacteria, virus or fungi consists of two functional parts, the innate and adaptive systems. Innate immunity forms the first line of defence, and if this defence is breached the adaptive system is activated and produces a specific response to each infectious agent, normally eliminating the infection. The innate immune system reacts fast to a pathogen but it lacks memory. The two key factors of the adaptive response are such specificity and memory. Good descriptions of these functions are given in textbooks of immunology such as Tak W. Mak and Mary E Saunders: The immune response. Basic and clinical principals. 2006, and Charles A. Janeway, Jr. and Paul Travers: Immunobiology. 2001.

2.1.1 Innate immunity

Innate immunity consists of defences that exist prior to antigen exposure and that are found in both invertebrates and vertebrates. Innate immune responses are mediated by receptors that are genetically programmed to recognize macromolecules shared by many organisms. Skin and mucosa with antiseptic substances on their surfaces as well as saliva and tear fluid are parts of the innate immune system, as are plasma proteins such as those comprising the complement system.
The innate leucocytes include: natural killer (NK) cells, mast cells, eosinophils, basophils; and several phagocytic cells including macrophages and neutrophils that identify and eliminate pathogens. Macrophages, mast cells, monocytes and granulocytes identify pathogens by recognising conserved molecular patterns shared by large groups of organisms such as the lipopolysacharide (LPS) of Gram-negative bacteria and lipoproteins of Gram-positive bacteria. Macrophages interact directly with microorganisms and release cytokines that activate neighboring cells (1).
Dendritic cells, present in the epithelia, are capable of antigen presentation. Immature dendritic cells act as sentinels that patrol the body. When guided by cytokines, or the Pathogen-Associated Molecular Patterns (PAMPS), they become highly effective in capturing antigens via phagocytosis, macropinocytosis, or endocytosis. Dendritic cells are capable of activating the adaptive immune system via interaction with Thymus-derived lymphocytes (T cells), but may also contribute to the development of peripheral tolerance via ingestion of apotopic bodies (2).

2.1.2 Adaptive (Acquired) immunity

Adaptive immunity, in contrast to innate immunity is only found in vertebrates and involves lymphocytes derived from the bone marrow (B cells) and T cells that are selected during the development of the immune system. The selection process ensures that these B and T cells are biased towards recognition of non-self antigens. In addition, the selection of highly specific clones allows the production of a small population of memory cells. The adaptive system has specificity to recognize individual pathogens and creates an immunological memory. It thus has the ability to react more effectively with each encounter of a specific pathogen. Adaptive responses build up gradually and reach their maximums intensity only after re-exposure to the antigen (3).
The antigen presenting cells (APCs), especially dendritic cells, that have captured the pathogen and processed the antigen migrate from the tissue to the T cell zone in the lymph node (2, 4).
There are two fundamental adaptive mechanisms: cell-mediated immunity (activated T cells) and humoral immunity (activated B cells). The adaptive response consists of T and B cells activated by APC in the lymph nodes draining the site of infection. Macrophages are less effective than dendritic cells in presenting antigens but can nevertheless induce T cell activation. T cells recognize antigen fragments

associated with molecules encoded by major histocompatibility complex (MHC), presented at the surface of cells of the body. Mature dendritic cells are potent T cell activators and stimulate CD4+T cells via MHC Class II antigens and CD8+ T cells via MHC Class I antigens. B cells produce antibodies that can recognize intact antigens.

2.1.3 The skin immune system

The skin is an active immunological microenvironment. The skin immune system has been defined as the cutaneous complexity of interaction of immune response-related cells. The skin-associated lymphoid tissues include keratinocytes, the epidermally localized dendritic cells named Langerhans cells (LCs), the skin-seeking T cells and the endothelial cells of the skin. The term skin immune system (SIS) is understood to describe the complexity of immune response-associated cells and humoral factors present in normal human skin (5).
T cells of human skin are present in a large number, around 4 billion, most of which localized in the dermal perivascular units. Many of the skin-specific features of the SIS are related to its superficial location and the fact that it is continuously challenged with antigen. The effector function of the immune system in the skin is a unique combination of pro-inflammatory stimuli from keratinocytes that prepare the dermal stroma for specific immunological activity, paralleled by a simultaneous increase in the migratory activity of antigen-trapping APCs (LCs), which induce expansion of specific lymphocytes in the skin-draining lymph nodes (5).

2.2 INFLAMMATION

Inflammation with the four cardinal symptoms rubor (redness), tumour (swelling), calor (heat) and dolor (pain) is the individual's reaction to trauma or to infection. It is initiated by the tissue damage *per se* or caused by a pathogen breaking through the immunological barriers. This activates the innate immune system and if the pathogen persists the adaptive immune system is also activated.

2.3 AUTOIMMUNITY AND AUTOIMMUNE DISEASE

Self tolerance means tolerance against the body´s own potentially antigenic substances. Autoimmunity is the response of the adaptive immunity towards self antigen, it encompassing activation of T and/or B cells towards self molecules, something that sometimes also leads to development of autoimmune disease. In 1902, Paul Ehrlich described the presence of autoreactive cells as a state of "Horror autotoxicus" and questioned the existence of autoimmunity. Subsequently, it has been demonstrated that some autoimmunity is part of the body´s normal homeostasis, and that autoimmune disease is a result of a harmful further activation of this autoimmunity. Thus many autoantibodies such as rheumatoid factor (RF), one of seven criteria in RA, exist normally in the population, although often only at a low titre and occurring more with increasing age.

Approximately 5% of the population in the Western world suffers from different autoimmune diseases. The spectrum of autoimmune diseases is broad, from organ-specific conditions such as Graves's disease and Type I Diabetes with autoantibodies against specific organs, to systemic autoimmune diseases with antinuclear antibodies (ANA) affecting many organ systems, such as Systemic Lupus Erythematosus (SLE). RA is also a systemic autoimmune disease, affecting many organs even if the main effected organ is the synovial joint.

2.4 RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) is a common disorder of connective tissue and is an important cause of disability, morbidity and mortality. Overall there is a 3:1 female preponderance, but this excess is greater in young people. The outcome of RA is difficult to predict. Some patients have a spontaneous remission but the most patients suffer for years, and their disease usually results in joint damage and loss of function.

2.4.1 Clinical and Laboratory features

RA is characterized by synovitis with symmetrical involvement of small as well as large joints. RA is a systemic disease affecting not only the joints and skeleton but also muscles, tendons, blood vessels, nerves and many organs such as skin, eye, lungs, heart, kidney, liver, spleen, gastrointestinal channel, spleen and the haematopoietic system. Thus, common results of the disease and/or the medication are osteoporosis, myalgia, tenditis, anaemia, haematoma, wounds, conjunctivitis sicca, and sometimes vasculitis, neuropathies, pneumonia and also heart disease, nephritis, amyloidosis, affection of the liver, or more seldom of the spleen.

The overall risk of developing solid cancers does not seem to be increased in RA. However, an increased risk to develop lung cancer and non-melanoma skin cancer has been reported (6) and also malignancies of the reticuloendotelial system such as lymphoma have been reported (6-9), and in two studies also multiple myeloma (10, 11).

Comorbidity is seen with other autoimmune diseases, such as Hashimoto's disease (12, 13) and pernicious anaemia (14). A number of disease-related autoantibodies have been discovered, one of which is recognized as a diagnostic criterion, Rheumatoid Factor (RF) discovered in 1937 by Waaler (15, 16). RF:s are autoantibodies against immunoglobulin G (IgG), and levels above a certain diagnostic level occur in about 60% of RA cases. Anticitrulline antibodies (anti-CCP) (17) seem to be a more specific autoantibody and have predictive ability for the development of RA. Anti-CCP may, as RF, occur before any symptoms of disease. ANA and other autoantibodies are also more common in RA than in healthy individuals.

2.4.2 Definition

The course is often insidious, initial symptoms are tiredness, morning stiffness and symmetrical joint aching and swelling, especially of the small joints of the hands and feet. Since 1987 the diagnostic criteria performed by the American college of Rheumatology (ACR) (18) are used.

1. Morning stiffness for at least 1 hour.
2. Arthritis of three or more joint areas simultaneously, including wrists, finger joints (except for the distal interphalangeal joints), elbows, knees, ankles and metatarso-phalangeal joints of the feet.
3. Arthritis of hand joints (except for the distal interphalangeal joints).
4. Symmetric arthritis.
5. Rheumatoid nodules.
6. Rheumatoid factor.
7. Radiographic changes – erosions or juxta-articular osteoporosis.

Patients fulfilling at least four of these seven criteria are classified as having RA. Criteria 1-4 must have been present for at least six weeks. Patients with two clinical diagnoses are not excluded.

2.4.3 Pathophysiology and pathogenesis

The primary targets of inflammation are synovial membranes and articular structures. Other organs are also affected. Inflammation, proliferation and degeneration typify synovial membrane involvement. Joint deformities and disability result from the erosion and destruction of articular surfaces and bone.

The normal joint

Synovial joints (or diarthroses) are the most common and most moveable type of joints in the body. The synovial joint is contained by a ligamentous sac, the articular capsule. The surfaces of the two bones are covered by cartilage. The synovium is a structure that covers the non-cartilaginous surfaces within the joint capsule. It secretes synovial fluid into the joint, which nourishes and lubricates the articular cartilage.

The RA joint

An inflamed synovium is central to the pathophysiology of RA. Histology reveals an influx of inflammatory leucocytes and changes in the expression of cell-surface adhesion molecules, proteases and many cytokines. Initially, tissue oedema and fibrin deposition are prominent and coincide with clinical symptoms such as swollenness of joints and pain. Within a short period after onset of disease, the synovial lining becomes hyperplastic, consisting of macrophage-like and fibroblast-like synoviocytes. Mononuclear cells including T cells and B cells, macrophages and plasma cells infiltrate the sublining. Synovial vessel endothelial cells transform into high endothelial venules that are specialized post-capillary venules found in secondary lymphoid tissue or inflamed non-lymphoid tissues; they facilate the transit of leucocytes from the bloodstream into tissues. Pannus, locally invasive synovial tissue involved in the joint erosions, is a hallmark of RA. Initially the pannus is composed of mononuclear cells and fibroblasts, later replaced by a fibrous pannus (19).
The most abundant cells in the synovial membrane are macrophages and T lymphocytes, but plasma cells, dendritic cells and activated fibroblasts are also present. Many of these cells are activated and express abundant HLA class II and adhesion molecules of relevance in antigen presentation (20). The destruction of the cartilage is considered to be mostly due to the activity of matrix metalloproteinases (MMPs), enzymes produced by activated macrophages and fibroblasts in response to proinflammatory cytokines such as interleukin (IL)-1 and Tumor necrosis factor alfa (TNF-α). In addition to MMPs other enzymes are active in the destruction of bone and cartilage (20).

Thus inciting agents activate the immune system resulting in immunological reactions; immune complexes in the synovial fluid activate complement and an early inflammatory response in the synovia with increased vascularity and increased production of synovial fluid with evidence of CD4+ T helper cells migrating into the joint. Early and intermediate molecular mediators of inflammation include TNF-α, IL-1, IL-6, IL-8, IL-15, Transforming growth factor beta (TGF-β), fibroblast growth factor and platelet-derived growth factor. Modern pharmacological treatment of RA targets these mediators. Once the inflammatory reaction is established, the synovium thickens, the cartilage and the underlying bone begin to disintegrate and evidence of joint destruction accrues.

As illustrated in fig 1 the RA synovium is characterized by a complex network comprising both immigrated and resident cells.
Macrophage-like cells are abundant in RA joints, both in the synovial membrane and the synovial fluid. They have an activated phenotype with high levels of HLA-DR expression. Synovial macrophages

represent the principal source of synovial cytokines and are important mediators of local inflammation and joint damage (21).

T cells are present in the RA synovium. The majority belonging to CD4 subtype, but there are also CD8+ T cells. According to the type of cytokines produced, a T cell is classified as being either a Th1 or Th2cell. It has been proposed that RA synovial T cells are biased towards a Th1 phenotype with almost no Th2 cytokine gene expression (22).

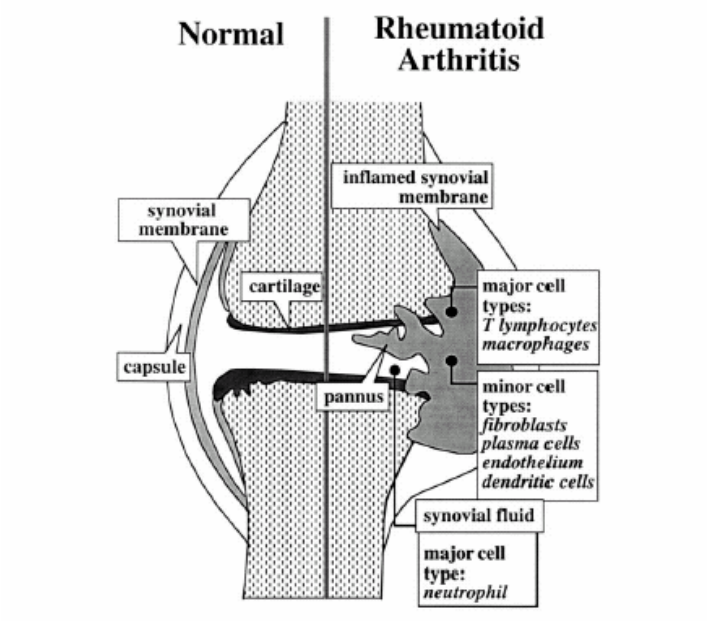
Fibroblast-like cells are non phagocytic cells. RA synovial fibroblast cells are able to secrete large amounts of hyaluronic acid, cytokines and arachidonic acid metabolites.

Dendritic cells function as potent antigen presenting cells

B cells and plasma cells are only a minority of the RA synovial cells, but they are of pathogenetic importance due to their capacity to secrete RF.

Neutrophils are mainly present in the synovial fluid and to lesser extent in the synovia.

Fig. 1



The rheumatoid joint

Schematic illustration of normal (left) and rheumatoid arthritic (right) joint. Adapted from Feldman (20)

A previous hypothesis has been that RA is a disease driven by T cells after an unknown initiating event. CD4+ T cells stimulate the immune cascade leading to production of cytokines such as TNF-α and IL-1. The persistent inflammation is maintained by autoantigens (23). RA synovitis is characterized by new blood vessel formation, thickening of the lining layer and an infiltrate comprising mainly of mononuclear cells consisting of HLA-DR positive APCs in close contact with T lymphocytes, belonging to the T helper 1(Th1) type.
However, there also is an interaction of the CD4+ T cells with B cells. Autoreactive B cells can be driven by the T cells to produce IgG autoantibodies that may be directly involved in joint damage (24).
Not only the adaptive immune system but also the innate immune system contributes in RA (25). The cellular and protein effectors of innate immunity are found in the synovium, and an increasing body of evidence indicates that they are directly involved in joint inflammation and in destruction of the joint cartilage and bone. In addition they may have regulatory effects on inflammation and immunity. Mature dendritic cells may indirectly modulate synovial inflammation in response to stimulation by a virus, shift the cytokine balance toward a proinflammatory or an anti-inflammatory pattern, or directly deviate the local immune response as a result of defective cytokine function. Cytokines are the forefront of cell-to-cell interactions. They stimulate the cells involved in innate immunity, and they enhance or inhibit inflammatory responses and adaptive immune responses. Many cytokines are involved in joint destruction, although TNF-α and IL-1 play a prominent role.

2.4.4 History

It is unclear how long RA has existed in the world. Indications of RA seem to appear in Europe during the last part of the 17th century, when described by the English physician Sydenham published in Latin in 1676 (26). However, the first real admitted description of RA was by Landré-Beauvais in Paris in 1800 (27). During the second half of the 19th century the name “rheumatoid arthritis” was introduced by Garrod who distinguished gout from chronic rheumatism using a thread test for demonstration of uric acid crystals (28).
RA-like destructive polyarthritis have been reported from North American archaeological findings skeletons dating from 1000-3000 years B.C. Observations of 13 cases of compatible RA lesions were found in 129 examined skeletons. As lesions compatible with a diagnosis of RA have not been noted in the Old World before 1785, Rothschild & Woods suggested the likelihood that RA is a New World disease which subsequently spread to the Old World. The slightly increased occurrence of RA in Archaic Indians is in keeping with the increased occurrence of RA in contemporary American Indians (compared with non-Indian populations) (29). However, whether or not RA existed in Europe in ancient times has been questioned and debated. In 1988 Leden presented results of an examination of skeletons found during an excavation of a Neolithic burial place in the island of Gotland, Sweden. In two skeletons there were changes compatible with the *in vivo* presence of a chronic aseptic polyarthritis (30). Nonetheless, not only in humans but also in other mammals in North America, RA-like destructive polyarthritis has been reported, such as in petroleum springs in North America where paleological arthritic findings of the sabre toothed cat can be seen by the public at the La Brea museum.

2.4.5 Occurrence

RA is a globally occurring disease, described in all ethnic groups. Studies conducted during the last three decades in Third World countries have confirmed that RA occurs throughout the world (31). It may be less severe in Asia and West Africa than in western countries. Today some Indian tribes (Pima and

Yakima) report a very high RA prevalence of 5-6%. RA is less common in certain rural societies in Africa, but more common when the inhabitants move to industrial cities (32-34). The prevalence of RA in Scandinavia has in recent years been estimated at about 0.5-0.8% (35-37). In 2006 Alamanos reported in a systematic review the incidence and prevalence of RA based on the 1987 ACR criteria. The occurrence of RA varies among countries. A decreasing trend has been observed in countries characterized by high rates of RA incidence and prevalence (38). Epidemiological studies of RA require a set of diagnostic-sensitive and -specific criteria that identify cases and which are easy to use in clinical practice. As besides the population must be known in number, age and gender, and have access to medical care, it is difficult to estimate incidence and prevalence in many countries.

2.4.6 Etiology

The pathogenesis of RA is a complex phenomenon. Factors associated with RA include the possibility of genetic predisposition, sex, environmental triggers including infections, and autoimmune responses.

2.4.6.1 Genetics

There is a strong genetic basis for RA (39-41). However, according to Lipsky "The genes that predispose to RA are normal ones that serve to alter the reactivity of the individual. Some genes control the responsiveness of the individual's innate or adaptive immune system and others likely control the intensity or the organ manifestations of RA" (42).
Both genetic heritability and environment contribute to the risk of developing RA. Twin studies of mono- and dizygotic twins indicate the importance of both genes and environment (43). Based on twin studies (43, 44), it was estimated that approximately 60% of the occurrence of RA in twins is explained by a shared genetic effect (45). Genes within the MHC are the most known of the genetic contributers (46). A linkage to HLA DR4 and also other HLA DR alleles has been reported (47, 48), with linkage to different DR alleles in different populations. Later it was hypothesised that these class II molecules share a common amino sequence, the shared epitope (SE), in one of the two chains of the molecule. This sequence, SE, of the DRβ1 chain resides in the peptide binding cleft and is considered to implicate the presentation of a particular antigen contributing to RA (39, 49). SE sequences shown to influence susceptibility to RA in patients in the UK also play a role in patients in Greece. However, 57% of Greek patients lack the putative HLA-DRβ motif, which suggests that considerable immunogenic heterogeneity underlies disease susceptibility in this population (50). The association with the HLA-DRB1 gene is the best understood, although several non-HLA loci have been linked to RA.
Additional candidate gene polymorphisms have been suggested to be linked to RA, but only relatively few have been successfully replicated (51). Such replicated genes include PTPN22 that encodes an intracellular protein tyrosine phosphatase involved in triggering leucocyte activation, including activation of MHC-II-dependent T cell activation (52). Recent data from genetic epidemiology, together with information on citrullination in the lungs of smokers, have suggested a new hypothesis for anti-CPP-positive RA, that smoking in HLA-DR SE positive individuals might trigger immunity to citrulline-modified proteins and later result in arthritis (53).

2.4.6.2 Sex hormones

Concerning the fact that female is the dominating sex affected by RA, many speculations about the role of sexual hormones have ensued. In 1978 the Royal College of General Practitioners' oral contraception study (54) published a paper in the Lancet of lower frequency of reporting of RA in oral-contraceptive

users than in non-users. The findings of an investigation by Allebeck could be compatible with the hypothesis that the use of oral contraceptives was associated with a reduced incidence of RA (55). In 1989 Spector and Hochberg, after an overview of the analytical epidemiological studies using meta-analysis, concluded that these results showed that oral contraceptive pills may not have a protective effect on the development of RA, but may rather prevent the progression from mild to severe disease by modifying the disease process (56). The hormonal or reproductive influence on RA incidence was limited in the Nurses Health Study (57). However, results regarding hormones are contradictory. Sex hormones have been considered as risk factors for the development of RA, as well as modifying factors, influencing the course of the disease (58). According to Doran, exposure to oral contraceptives, but not estrogen replacement, significantly reduced the risk of development of RA (59). Decreased levels of male sex hormones, androgens, and increased levels of the female sex hormone oestrogen have been reported in RA patients of both sexes (60). Recently in a large cohort, breast-feeding >12 months was inversely related to the development of RA. However, other reproductive hormonal factors were not associated with RA risk (57). According to Butts and Sternberg: it is important to note that gender-specific differences may also be related to specific agents, which can serve as initiators of disease, differences in gene expression, and behaviours (61).

2.4.6.3 Environment

Two known risk factors for RA are silica and smoking. They will be discussed below as the role of socioeconomic classes, occupation, and diet and immunisation. Infectious agents have also been hypothesised to initiate RA, but data in this regard are contradictory.

2.4.6.3.1 Silicon compounds

Silica

Silica is a mineral, consisting of SiO_2, one of the most common of the earth crust. Occupational exposure to silica was identified as a risk factor for RA in 1987, when the prevalence of RA was found to be higher among granite workers than in the general male population (62). Recently in the EIRA study it was shown that men who had worked with rock drilling or stone crushing had a threefold increased risk of RA (63).

Silicone

Silicone is the generic term of inorganic polymers based of chains of silicon and oxygen. In 1964 Miyoshi et al first described human adjuvant disease; two cases with hyperglobulinemia following injection of paraffin with improvement of signs and symptoms following mastectomy (64). Kumagai described a series of 46 patients with signs and symptoms of connective disease, including RA, following injection of foreign substances, either paraffin or silicone (65). In 1982 Van Nunen et al described a chronic autoimmune syndrome (SLE, RA and scleroderma) in three patients following mammary augmentation with gel-filled type prostheses (66, 67). Four cases of scleroderma, arthritis, RF and ANA following breast augmentation were reported in 1987 (68). Silicone gel from breast implants has been suggested to be an adjuvant (69). However, silicone gel did not appear to be arthritogenic in later experiments (70). Besides, later epidemiological investigations have failed to support any association between breast implants and connective tissue disease (71, 72). Additionally, on the basis of a meta-analyses in 2000 there was no evidence of an association between silicone-gel-filled breasts implants and any of the individual connective tissue diseases or rheumatic conditions (73).

2.4.6.3.2 Smoking

Positive rheumatoid factor (RF) reactions commonly precede the onset of RA. In 1990 Tuomi suggested an impact of smoking on RF production and in a follow-up study that raised RF titers in smokers would be reflected as an increased incidence of RA (74).
However, already in 1987 Vessey *et al* had found a strong association between referral to hospital for RA and cigarette smoking (75). Since then cigarette smoking has been one of the most important studied potential lifestyle risk factors. Male smokers are almost three times more likely to develop RA and heavy smokers even more (76). Uhlig found that male smokers were more likely to develop RA, but there was no significant increased risk for female smokers (77). One study even observed female smokers to be less likely to develop RA than female non-smokers (78). Other studies have investigated women separately and determined a correlation between smoking and increased risk of RA (79-81). A stronger association with smoking has been found for RF-positive RA (77, 81) and in some studies only an association with seropositive RA (77, 82) as in the EIRA study (83). The increased risk of RA associated with smoking was observed to require a long duration of smoking (80, 82). A relationship between the risk of RA and the cumulative dose of smoking was evident in the EIRA study (83).
A gene-environment interaction between smoking and SE genes in HLA-DR, providing a high risk of seropositive RA, was reported in an EIRA-study in 2004 (84). Recently, another EIRA study proposed a biological basis for smoking in the aetiology of RA, with the suggestion that smoking in the presence of HLA-DR SE genes may trigger RA-specific reactions to citrullinated proteins (85).

2.4.6.3.3 Socio-economic class

In 1970 Allander in a population survey of rheumatoid arthritis pointed out "One striking feature is the significantly lower proportion of RA males and females with higher secondary school or university education in comparison with the contrast group. RA persons were underrepresented in more qualified occupations." (86). This is in accordance with the observations of Engel *et al* (87) and Adler & Abramson (88) and also with recent results from the Swedish EIRA study (89). However, a major population-based English study showed no evidence of increasing incidence with decreasing social class (90).

2.4.6.3.4 Occupation

In 1970 Hellgren undertook an extensive study of occupation in relation to RA (91). A total of 39418 persons were investigated in a sample survey of 5 geographical areas. All persons were examined and the Rome criteria for active disease were used. Hospital records and X-ray documents were consulted. The completion rate was 80-90%. There were many occupational groups with a high prevalence of RA, but after adjustment using standard indices to correct for among others age, the highest index for classical arthritis was found in mariners and in female gardeners; next came nursing assistants, farmers and male foremen. In a meta-analysis published in 2002, a significantly increased risk was seen for hairdressers with both sexes included in the analyses (92).

2.4.6.3.5 Diet

The possibility that diet may play a role in RA has frequently been discussed. However, there are a number of problems associated with studying diet. Evidence exists that diet may play a role in the etiology of RA, but it is inconclusive due to the small number of studies available and variation in study design (93).

2.4.6.3.6 Infection

The greatest interest in an environmental cause for RA is based on the hypothesis that infection is the initiator of the inflammatory process (94, 95). Bacteria and viruses have long been incriminated in the pathophysiology of noninfectious chronic arthritis, although proof of a direct role has not been obtained (94). Several viruses as parvovirus, Epstein-Barr virus and bacteria such as Streptococcus and mycoplasma have been discussed. A population-based systematic survey of the infectious background of patients with early synovitis (duration of symptoms <3 months) was performed by Söderlin (96). Seventy-one patients were included in the study, 45% of them having had a prior infection. However, most of the patients with a recent infection and undifferentiated arthritis went in remission during the first 6 months. Two patients who fulfilled the criteria for RA had serologically verified recent infections, one with parvovirus B19 and the other with *Chlamydia pneumoniae*.

2.4.6.3.7 Immunisation

In 1997 Harrison reported that 'Patients who develop inflammatory polyarthritis after immunization are clinically indistinguishable from other patients with inflammatory polyarthritis' (97). Results from a case control study suggested that the frequency of prior immunization was higher among cases (5.5%) compared to age and sex-matched controls (2.8%): OR 1.7 (0.5-5.4). Whereas this supported the suggestion of an association between immunization and inflammatory polyarthritis, the results were not conclusive. The question has been asked whether immunization can trigger RA. Rubella vaccine is the only vaccine for which there has been evidence of a link with arthritis (98), but in both women and children the risk of arthritis following rubella immunisation was less than after natural immunisation with wild rubella virus (99).

2.5 IMMUNISATION AND IMMUNOLOGICAL ADJUVANT ABILITY

For a long time, in immunisation and vaccination, different adjuvants have been used to strengthen the immune effect of the bacteria or virus. With an adjuvant you can use low concentration, denatured or killed bacteria or virus. The term 'adjuvant' from Latin: adjuvare, to help, has been used for any material that can increase the humoral or cellular immune response to antigen. An immunogen consists of an antigenic part (epitope) and an adjuvant part (100). The tested adjuvants were shown to induce more marked and prolonged circulation of lymphocytes through draining lymphoid tissue, with increased trapping of lymphocytes than do weak immunogens (101). According to Dresser all adjuvants abrogate tolerance and enhance immunity (102). The complexity of adjuvant effects has been underestimated (103). There are many substances with adjuvant activity, most known being silica, aluminium salts and oils, particularly mineral oils. Many bacteria also have an adjuvant effect and have been used to strengthen the effect by immunization. Bacillus Calmette-Guerin (BCG) was used to enhance the immunological response during oncological treatment of patients.

2.5.1 History

In the beginning of the vaccination era 200 years ago the contents of cow-pox were mixed in milk, cream or butter. From experience it was known that with the mixed infectious agent in a lipid you get a better

response to immunization. In 1897 it was found by Rabinovitsch in Koch's laboratory that living *Mycobacterium butyricum* injected into experimental animals caused lesions at the sites of injection, sometimes resembling tubercles, but when the bacteria were incorporated into butter they evoked a cellular reaction almost identical with tubercles caused by pathogenic tubercle bacilli. In 1899 Grassberger reported that paraffin oil was far more effective (104).

The first known scientific report of use of an oil emulsion as adjuvant was in 1916 when Le Moignic and Pinoy found that a suspension of killed *Salmonella typhimurium* in mineral oil increased the immune response (105). In 1925 Ramon first demonstrated that it was possible artificially to increase levels of diphteria or tetanus antitoxin by addition of bread crumbs, agar, taipioca, starch oil, lecithin or saponin to vaccines. Ramon introduced the concept of adjuvant and immunostimulating substances (106).

2.5.2 Adjuvant substances

The following substances have been described as possessing activity in enhancing the immunogenicity of various antigens when injected at the same time and together with the immunogen: alginate, saponin, lanoline, phospholipids, methylcellulose, quaternary ammonium compounds, vitamin A, silica, and several bacteria, exotoxins from staphylococci (107) (Table 1). Quite a lot of substances have been in use in vaccination. Many adjuvants occur among the pharmaceutical emulsifying agents, which are surface-active substances capable of stabilizing an oil-water interface. Adjuvants are often made up of separate lipophilic and hydrophilic moieties, which is what makes them surface-active (100). In the 1990s, several reports have been published on oil-in-water emulsions, particularly squalene (108).

2.5.3 Freund's adjuvant

The development of emulsified mineral-oil adjuvants emerged from studies of tuberculosis. Freund noted a remarkable increase in both antibody and delayed hypersensitivity response to killed mycobacteria if the organisms were incorporated in paraffin (mineral) oil (109). In 1947 Freund developed Freund's complete adjuvant (FCA), containing mycobacteria, mineral oil and emulsifier, and Freund's incomplete adjuvant (FIA) containing only mineral oil and emulsifier, which are still two of the most used adjuvants in the laboratory (110). FCA increases both the cell-mediated and the humoral immunity antibody concentration, and increases the activity of the reticuloendothelial system. FIA increases the antibody concentration (111).

Mode of action

Even if it is now more than 50 years ago since Freund described the mode of action of adjuvant (104) it is still poorly understood. Adjuvants exert their effects in several ways such as slowing the release of antigen. The lipid of the adjuvant is shortly after injection demonstrable in the lymph nodes where it stays for a long time and where the activation of antigen-specific cells takes place. The n-hexadecane component and most likely other straight-chain hydrocarbon components of mineral oil adjuvant emulsion are very slowly mobilized from the site of injection in monkeys and rats (112).

The adjuvant effects are complex. There is a much greater variety of cells than originally envisaged upon which adjuvants may act: Auxiliary cells (macrophages, LCs, dendritic reticulum cells), the many varieties of T cells (helper, specific and nonspecific suppressor, cytotoxic) and at least two types of B cells (113).

FCA is a known stimulator of the Th1 phenotype in contrast to alum that stimulates the Th2 phenotype.

2.5.4 Adverse effects

Administration of FCA alone to animals has been found to cause various pathological disorders such as adjuvant induced polyarthritis in rats, amyloidosis in mice (114) and generalized granulomatous lesions in several species (115), and also glomerulonephritis (116). Because of adverse effects such as severe pain, abscess formation, fever and the possibility of permanent organ injury following the use of this adjuvant, FCA can be used only for experimental purposes and not in humans or veterinary vaccines any more. In the 1970s the minimal structure needed for adjuvanticity of FCA was defined as being N- acetyl muramyl-L-alanyl-D-isoglutamine, commonly known as MDP. MDP itself was still too toxic, especially pyrogenic, for human use. Reported side-effects of MDP, among others, are adjuvant arthritis in rats (105). Systemic effects have been reported of FCA and FIA with fever, tiredness, granulomas at the site of injection and also carcinogenic effects, among others multiple myeloma. When working with FCA and FIA precautions must be taken to not be injured by contaminated needles. Mineral oil is used as a means of inducing multiple myelomas in mice and it may, therefore, be more than coincidence that Freund himself, who developed the most used adjuvant, died from multiple myeloma (117).
Previously, FIA has been used in human immunisation. The mineral oil most commonly used in humans was a highly complex mixture of normal, cyclic, and branched-chain hydrocarbons and aromatics (118). Arlacel A, a heterogeneous complex of poorly defined fat and carbohydrate compounds plus mannide mono-oleate, is employed as emulsifier (118). Salk and Laurent used a mineral oil emulsion in their serological experiments with monkeys (119). The oily vehicle also served in the active immunization of human subjects against poliomyelitis (120). Further improvements in polio immunization employing live vaccines made the use of mineral oil unnecessary for this purpose, but in influenza and particularly in adenovirus vaccinations (121), this adjuvant found fairly wide clinical application (115).
In 1963 an oil-adjuvant influenza vaccine was approved in the United Kingdom and Freund's adjuvant was licensed for use with influenza vaccine. Observations of severe local reactions discredited its use in all subjects except persons with a high risk of death from influenza (122). However, the incidence of delayed local reactions was too low to affect the widespread use of FIA in influenza vaccine in the US armed forces. Anyhow, no increased tumour formation could be detected in 18,000 recipients of FIA influenza vaccines followed for 18 years (123, 124). The 10-and 18-year follow-up studies of persons who received mineral-oil influenza vaccines might not be sufficiently long to absolve oncogenic or autoimmune effects of the vaccine in humans (125).

Toxic oil syndrome is a syndrome with arthralgia /arthritis and features overlapping those of various forms of scleroderma (126). An epidemiological investigation has linked the occurrence of illness with ingestion of unlabeled, illegally marketed cooking oil. Severe neuromuscular manifestations (myalgia severe enough to restrict movement, motor deficits, atrophy of major muscle groups, and contractures of the jaw and extremities) occurred late in the illness in 23% of the patients (127).

2.5.5 Autoantibodies

Burnet speculated that the production of autoantibodies by experimental immunization might occur only with the use of unnatural procedures such as the addition of Freund's adjuvant (128). It was assumed that induction of autoantibodies as well as triggering of autoimmune disease was permitted preferentially after

addition of adjuvants, which would create local compartments where immune responses toward foreign as well as self antigens would be enhanced and be subject to less control by the thymus (129, 130). However, later studies have shown that immunity to self antigens is also a common and physiological phenomenon in the absence of adjuvants (131). Nonetheless, addition of adjuvants greatly enhances immunity against all types of antigens including autoimmunity that may contribute to autoimmune disease (132). Although mineral oils are generally considered nontoxic and have a long history of use in humans, the mineral oil Bayol F (FIA) and certain mineral oil components (squalene and n-dexadecane) induce lupus-related anti RNP/Sm or –Su autoantibodies in non-autoimmune mice. Kuroda induced autoantibodies by using mineral oils considered nontoxic and thought that this also may have an implication in human autoimmune diseases. FIA mainly contains C15-C25 hydrocarbons; MO-F (a medicinal mineral oil) contains C15-C40. Anticytoplasmic antibodies were common in mice treated with pristane, squalene and FIA (133). Induction of these autoantibodies appeared to be associated with the hydrocarbon's ability to induce IL-12, IL-6 and TNFα, suggesting a relationship with the hydrocarbon's adjuvanticity. Whether this was relevant in human vaccination was a difficult issue due to the complex effects of vaccines and the fact that immunotoxicological effects vary depending on species, route of administration and dosage (134).

2.5.6 Immunoglobulin subclass

Adjuvants influence the immunoglobulin subclass distribution of immune responses *in vivo*. In most species FCA favours the synthesis of IgG. FCA stimulates production of IgG1 and IgG2 antibodies, whereas FIA immunized animals produce almost entirely IgG1 antibody (135). Addition of FCA or FIC led to IgG1 antibodies and the use of LPS as an adjuvant in the induction of both IgM and IgG, particularly IgG3 and IgG2b subclasses in a trial with mice immunized against fluorescein isothiocyanate (FITC)-labelled human gammaglobulin (HGG) (136). Intraperitoneal immunization of rabbits with alum-precipitated antigens enhances the production of IgE antibody, whereas immunization with FCA enhances IgG (135).

2.6 ANIMAL MODELS OF AUTOIMMUNE DISEASE AND ARTHRITIS

When inducing experimental arthritis with erosions the adjuvant is necessary. The method has been used for a long time for study of the inflammatory process and for measure of the effect of different anti-inflammatory and anti-rheumatic drugs. As said by Janeway: "In immunization in order to obtain readily detectable response to these antigens, they must be incorporated into a remarkable mixture termed complete Freund's adjuvant, heavily laced with killed *Mycobacterium tuberculosis* organisms or precipitated in alum and mixed with dead *Bordetella pertussis* organisms. I call this the immunologist's dirty little secret." (137).

Experimental chronic polyarthritis was described in 1954 by Stoerk and Budzilovich who injected rats with homologus spleen and Freund's adjuvants (138). In 1956 Pearson induced erosive RA-like polyarthritis in the rat with Mycobacteria suspended in adjuvant oil (139). However, experimental models for most autoimmune diseases can be induced in many mammals (140, 141) such as rat, mice, guinea pig, rabbit and monkey, among others, by inoculation of FCA and proper antigen using different routes of

administration (Table 2). Rat models are useful for studies of the pathogenesis of RA since rats are extraordinarily sensitive to induction of arthritis with adjuvant and several models have been developed (Table 3). For practical and economic reasons the most commonly used animals are rat and mice. The Dark Agouti (DA) rat is very sensitive and develops RA-like arthritis about 2-3 weeks after immunisation.

2.6.1 Arthritogenic substances

Whitehouse examined over a hundred compounds and natural materials for their ability to induce arthritis in rats when mixed with heat-killed delipidated *Mycobacteria tuberculosis*. Cyclization and/or the presence of oxygen atoms, or double bonds reduced (or abolished) the arhritogenic potential and adjuvanticity of alkanes>$C_{10.}$ Ester triglycerides of fatty acids >$C_{12,}$ retinol acetate and vitamins E and K showed co-arthritogenicity and adjuvant activity (142).

Concerning arthritogenic substances it is notable that single atom differences in the structure of linear alkanes can determine their capacity to induce arthritis, C16 H34 and C17 H36 being arthritogenic, while C16 H32 and C16 H33Br are not arthritogenic (143).

Pristane (2,6,10,14 tetramethylpentadecane) is of biogenic origin but is also a typical component of many mineral oils. Commercial pristane is extracted from shark and whale livers. Pristane injected intraperitoneally into mice induces an inflammatory seropositve arthritis in susceptible strains (144). Models like pristane-induced arthritis in rats are interesting models for RA, since they fulfil the RA criteria including a chronic relapsing disease course (145). Injection of not only FCA but also with pure adjuvants such as pristane and squalene without mycobacteria induce severe arthritis in many rat strains (145).

3 MINERAL OIL

3.1 ORIGIN AND HISTORY OF MINERAL OILS

Petroleum (from Greek *petra* - rock and Latin *oleum* –oil), crude oil or mineral oil are dark liquids found in porous rock formations in the earth. It is composed mainly of mixtures of chemical compounds of hydrocarbons, largely of the alkane series, with or without other non-metallic elements such as sulphur, oxygen and nitrogen. Mineral oils are chemically non-fats – they do not contain fatty acids or glycerine. Most geologists view crude oil as the product of compression and heating of ancient organic materials formed from the preserved remains of prehistoric zooplankton and algae which have been settled to the sea bottom under anoxic conditions. Because most hydrocarbons are lighter than rock or water, these sometimes migrate upward through adjacent rock layers until they become trapped beneath impermeable rocks, within porous rocks called reservoirs.

From history is known that the Egyptians used asphalt in embalming. More than four thousand years ago asphalt was employed in the construction of the towers of Babylon. The first oil wells were drilled in China in the 4th century. In the Middle Ages Marco Polo told about the occurrence of petroleum in Armenia. The American Indians waterproofed their canoes with mineral oil. In the beginning of the 17th century petroleum occurrence was noted in North America. At that time oil was used as a medicine and only to a small degree for lighting purposes. The oil industry grew in the 18th century, driven by the demand for oil lamps, and the introduction of the internal combustion engine in the early parts of the 20th century has further increased the demand. Today petroleum is mainly extracted by drilling, only exceptional petroleum springs being found (Wikipedia).

3.2 TYPE OF OIL AND OCCURRENCE

Today base oil is either produced from crude oil in a traditional way called “virgin base oil” or refined from recycled used oil (146). Base oil can be divided into four groups

1 Paraffinic base oils CnH_{2n+2} containing paraffinic hydrocarbons. Occurrence in Pennsylvania and Ohio in North America. Give good petrol and paraffin oil and a low proportion of asphalt.

2 Naphten base oils CnH_{2n} containing naphtenic hydrocarbons. Occurrence in Russia, Rumania. Give good petrol.

3 Asphalt base oils are very viscous. Occurrence in Mexico, California, and Venezuela.

4 Mixed base oils. A mixture of 1, 2 and 3. Occurrence in Mexico, Kansas, Oklahoma, Iran and Irak.

3.3 CLASSIFICATION

The oil industry classifies “crude” by the location of its origin and often by its relative weight or viscosity (“light”, “intermediate” or “heavy”); refiners may also refer to it as “sweet”, which means it contains relatively little sulphur, or as “sour”, which means it contains substantial amounts of sulphur and requires more refining. Each crude oil has unique molecular characteristics which are understood by the use of crude oil assay analysis in petroleum laboratories (Wikipedia).

3.4 FRACTIONS, NAMES AND USES

Petroleum or crude oil is practically useless as it is and has to be refined. Crude oils, from which lubricating mineral oils are obtained, contain thousands of compounds, some of which remain after the refining processes. The major mineral oil components are paraffinic, naphthenic, and a smaller amount of aromatic compounds. The variations in composition directly affect lubricant performance. Most commercial lubricants are mixtures where the composition is chosen for the proposed use. First the petroleum oil is distilled and fractionated into fractions.

Main different mineral oil products:

Petrol, paraffin oil and heavy petrol are used for solvents, pesticides; gas oil for diesel oil and for heating.
Vaseline oil is used for paraffin liquid, cosmetic oils, caulking, lubricating of finer instruments. Thin oil (spindle oil) is used as cooling oil and lubricating oil. Moderate thin oil is used as machinery oil.
Thick oil is used as machinery oil and motor oil.
Very thick oil used as cylinder oil, and paraffin - for candles, insulating material, and synthetic beeswax.
Residue is asphalt-petrolatum - for road asphalt, waterproof material, for unguents and creams.

Different types of oil are named depending on type of fraction, chemical properties or coming usage. The vocabulary is often confusing as the same word has different meaning in different countries. One example, the Swedish word “fotogen” is translated to “petroleum” in many languages, but in English to “paraffin” or “kerosene”. English “petrol” is “gasoline” in USA. “Mineral oil” in USA often stands for mineral oil laxative.

Here follow some common names and products (Science and Technology Encyclopedia):

Gasoline (motor fuel) (number of C atoms (Cn 5-12)) is a complex mixture of hydrocarbons that boils below 200°C and is intended for most spark-ignition engines.

Petroleum naphtha (Cn 5-12, boiling point 50-200°C) used for solvents.

Kerosine (Cn: 11-18, boiling point 175-250). Kerosine is used as a fuel for heating and cooking, jet engines, and lamps, for weed burning, and as a base for insecticides.

Diesel fuel (Cn >15) is a distillate product that has a higher boiling point than gasoline (or naphtha). Diesel fuel oil is essentially the same as furnace fuel oil, but the proportion of cracked gas oil is usually less.

Domestic fuel oil is used primarily in the home, and includes kerosine, stove oil, and furnace fuel oil.

Heavy fuel oil includes a variety of oils, ranging from distillates to residual oils that must be heated to 260°C or higher before they can be used.

Petroleum coke is the residue left by the noncatalytic destructive distillation of petroleum residua.

Medicinal white oil is an extremely pour boundary lubricant often used as a reference, primarily for research. However, medicinal white oil is used in food machinery lubricants where one may run a risk of ingestion by oral contamination.

Hydraulic oils or more specific hydraulic fluids are a large group of liquids made of many kinds of chemicals. Mineral oil is one of the three most common types of hydraulic fluids. The most common fluids are petroleum oils, synthetic lubricants, oil-water emulsions, and water-glycol mixtures. However, in recent years increasing reports of pollution effects on the marine milieu have started the increasing replacement of mineral oil by vegetable based oils, as soybean- based oils, when it is possible. Hydraulic

fluid serves as the power transmission medium in a hydraulic system. They are used in automobile automatic transmissions, brakes, tractors, bulldozers, industrial machinery and airplanes.

3.5 EXPOSURE

Petrochemical products have spread all over the world with industrialism and became an increasing part of modern human life, with exposure to petrochemical products in most situations of life. Mineral oil has been a cheap and rapid substitute to more expensive previously used animal or vegetable products. Besides flowing gas and oil products in transport systems petrochemical products are ingredients in house building materials, furniture, paints, and synthetic materials in clothes. Polishes for households, shoes, furniture, houses and cars often contain petroleum solvents and mineral oil as waterproofing materials. There is exposure to the skin by different cosmetic products, hair conditioners, and to mucous membranes by toothpaste, chewing gum and suppositories. There may be contamination of our food and sometimes even a conscious preparation of some food products with mineral oils for example polishing of chocolate and fruit as treatment of the skin of apples, so they will be more shiny and better resist hard treatment during transport. Inhalation of diesel products and other aerosols, gases emission after paints, glue, building of houses, leakage from machines and cars, discharge from airplanes and boats.

Referring to Whitehouse MW. “Possible hazards of adjuvants: While the aetiology of many autoallergic disorders still remains unknown it may be of some value to enquire if the average man is not unduly exposed to adjuvant-like immunostimulation from his everyday environment. Many materials with adjuvant activity are brought into contact with the body’s surfaces or the gastrointestinal tract, not only in processed foodstuffs and cosmetics, but also during clinical treatment by the use of poultices, laxatives, suppositories and massive vitamin supplementation. Furthermore, the increasing burden of petroleum products in the environment such as greases or oil droplets (in insecticidal sprays) also should be considered, since these also must accumulate on the skin. In combination with many bacterial and fungal cell walls, they may become potential adjuvants, for either boosting non-specific host resistance on the one hand or triggering autoallergy on the other, if they should happen to penetrate the external integument.” (147).

4 COSMETICS

4.1 DEFINITION AND REGULATION

The definition of cosmetics according to the European Union*: A cosmetic product shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition (148).
Cosmetic products are regulated both in Europe and North America and are distinguished from chemical products, food drugs and nature-cure medicines. The decree of cosmetic products is regulated by law in Sweden since 1999, but already in 1993 the Swedish definition of cosmetics was the same. Cosmetic products include moisturizers, creams, body-lotions, hair conditioners, hair dyes, soaps, nail cosmetics, protecting creams, plasters deodorants, eye cosmetics, baby oils, tooth paste, hair shampoos, permanent waving products, perfumes, powders, shaving creams and lotions, suntan preparations, vitamin creams, petrolatum for protection in connection with permanent waving and straightening of hair.

4.2 INGREDIENTS

Previously skincare products were often manufactured locally of animal fats or vegetable oils. Nowadays with big factories, centralized production and long transportation the animal fats or vegetable oils have often been replaced by mineral oils that have the advantage of being long-lasting, having no smell and low costs. Thus mineral oil is often an ingredient in skin care products such as body lotions, skin creams and ointments, but the exact composition is many times an industrial secret.
To get better penetration into the skin through the defending lipid layer a solvent is often used in moisturizers, skin creams and body lotions. Hair shampoos often contain different surfactants (anionic, non-ionic, amphoteric surfactants) (149).

4.3 ADVERSE EFFECTS

Adverse effects of cosmetics have been discussed for more than 100 years (150, 151). Lead in face powder and hair dyes caused anemia and lead palsy in the 18^{th} and 19^{th} centuries. Mercury in skin bleachers caused poisoning (152). That substances on the skin can penetrate the skin is known from history and experiments and this knowledge is applied practically in drug pharmacology (e.g. nicotine-, estrogen- and analgesic-containing plasters).
Today allergic reactions are common reported adverse effects caused by cosmetic ingredients such as fragrances and preservatives (153).

5 AIMS OF THE THESIS

The general aim of this thesis was to investigate the role of mineral oil as an immunological adjuvant in the development of RA.
However, I see it is a huge task to try to see the forest in spite of all the trees. As mineral oil and petrochemical products surround us everywhere, in the sea, in the earth, in the air, outdoors and indoors, I have chosen to concentrate on following questions.

1. To investigate if cosmetic products containing mineral oil can induce arthritis in certain rat strains.
2. To investigate if human occupational exposure to mineral oil is associated with increased risk of developing RA.
3. To investigate if human exposure to cosmetics that may contain mineral oil is associated with increased risk of developing RA.

6 MATERIALS AND METHODS

Here follows a summary of the methods employed for the completion of this thesis. Further details are found in the methods sections of papers I-IV, as indicated by their Roman numerals.

6.1 ANIMAL STUDY, EXPERIMENTAL

Paper I

Animals

Inbred DA rats of both sex and female Lewis rats at 2-5 months of age. The two rat strains share the same MHC haplotype but differ in background genes; only DA rats develop arthritis upon immunization with FIA.

Treatments

FCA, FIA, and two different mineral oils classified as medicinal white oil for food, pharmaceutical, and cosmetic use. Seven different commercial skin products containing possible immunological adjuvant (especially mineral oil): three skin creams, two body lotions, two baby oils, and one vaginal gel rich in mineral oil content. Silicone gel and a silicone elastomer envelope were taken from an unused sterile silicone mammary implant.

Routes of administration

Intradermal injections were given in the base of the tail with FIA, pure mineral oil and eight different common commercial cosmetic products containing a high degree of mineral oil. The negative controls received no injection. The animals were examined for clinical manifestations for 1-3 months. Note: This immunisation procedure was customary and approved by the animal ethics board at the time this experiment was performed. This intradermal immunisation procedure is no longer in use.

Percutaneous treatment in the shoulder region, repeated 10 times, on shaved and light abrased skin with baby oil, containing a high share of mineral oil. Control animals were shaved and abrased only. The animals were followed for 1 month.

Oral feeding was performed with pure mineral oil daily for 5 days. The animals were examined for clinical manifestations for 7 months.

Intravaginal treatment was performed initially with one FCA application followed by five applications of FIA during 12 days. The animals were examined for 3 months.

Subcutaneous implantations of silicone gel and silicone elastomer from human breast silicone implant were made. Two control animals were sham operated. The animals were examined for clinical manifestations for 6 months.

Examination and evaluation of joint inflammation

Animals were examined daily during the second week for clinical manifestations and onset of arthritis (and thereafter two to three times every week during the first months). Severity of arthritic joints was scored using a scale of 1-4 for each paws (maximum possible score of 16 per rat).

Skin irritation test

Six DA rats were shaved in the shoulder region and Baby oil was administered onto the skin, covered with a cotton compress, and fixed with a bandage for 24 hours. Two control rats were shaved and only covered with a bandage. Macroscopic inspection of the dermis was performed after 24 hr and skin biopsies taken.

Blood analysis

Retroorbital bleeding was performed. Note: This blood sampling procedure was customary and approved by the animal ethics board at the time of experimentation. Other procedures are currently in use for blood sampling from rodents.

Fibrinogen levels were determined in rats treated with silicone. Examination for autoantibodies was performed without knowledge of the treatment. Indirect immunofluorescence and ELISA were used to screen for organ-specific autoantibodies, ANA, RF, and antibodies to type II collagen.

Histology

Lymph nodes, spleens and ankle joints of baby oil treated animals were sectioned and stained.

6.2 HUMAN STUDIES, OBSERVATIONAL

6.2.1 Register-based cohort study

Paper II

Study population

Sweden has a long tradition of registration. National Registration Number (NRN); a unique ten digit number assigned each resident in Sweden. In 1964 the Swedish Hospital Discharge Register (HDR) started. Twenty of the County Councils reported inpatient care to HDR in 1983. The discharge diagnosis consists of one principle diagnosis and up to five contributory diagnoses coded according to a modified International Classification of Diseases (ICD).

By using registers a study was performed including persons born in 1905-1945 and living in 13 of 24 counties in Sweden in 1980. To be included they should have reported the same occupation in the census of both 1960 and 1970. The study population comprised a total of 375,035 men and 140,139 women and was followed concerning hospital care for RA in 1981-1983 by linkage to the Swedish HDR. Of those included 896 men and 629 women had been treated for RA.

Exposure

Each subject in the study population had reported the same occupation in the 1960 and the 1970 census. Thus the subject had probably been exposed to the same work environment for at least 10 years. In order to provide some information on the relationship between specific chemical exposures in the working environment, the occupations were classified in a job-exposure matrix (JEM) as exposed or non-exposed to a number of chemicals.

Statistical methods

The cumulative incidence of hospitalisation due to RA was compared between different occupations, and between different exposure groups based on the JEM. The comparisons and relative risk (RR) were determined according to Mantel and Haentszel with 95% confidence interval. Persons classified as exposed were compared to unexposed with adjustment for sex, age (stratified in 5 years categories) and residence (counties and degree of urbanization).

6.2.2 Population-based case-control study

Papers III and IV

Papers III and IV are based on the EIRA study which is a population-based case-control study of incident cases of RA among the population aged 18-70 years living in the middle and southern parts of Sweden during the period of May 1996 – December 2003. A case was defined as a person in the study base who for the first time received a diagnosis of RA according to the ACR criteria. The cases were included from 19 participating rheumatology centres. For each potential case a control was randomly selected from the study base with consideration taken for age, gender and residential area. The selection of controls was conducted using the national population register. Cases and matched controls answered identical questionnaires. The questionnaire contained questions within a wide

spectrum regarding personal circumstances, including lifestyle factors, occupational exposures, health aspects and socioeconomic factors. Specific questions were asked about occupational exposure to different mineral oils and about the usage of skin care products.

For each case the time point at which symptoms giving suspicion of RA started was used as an estimation of the disease onset. The year in which this time point occurred was defined as the index year. Only data on exposures up to the index year were analysed.

Blood samples for genotyping, serology and other laboratory investigations have been accumulated from cases as well as controls. Analyses were made for RF, anti-CCP and genotyping were made in respect of SE genes.

Exposed subjects were compared to non-exposed (respectively low-exposed in paper 4). Odd ratios, together with 95% confidence interval were calculated by means of logistic regression. We performed matched as well as unmatched analyses of the data. Odd ratios, adjusted for potential confounding factors, were calculated by means of conditional logistic regression in the matched analyses and by means of unconditional logistic regression in the unmatched analyses. Only results from the unmatched analyses are presented as these were in close agreement with those from the matched analyses but, in general, had higher precision. Odd ratios were interpreted as estimates of relative risks (RR) as the study was population based (154). Statistical analyses were performed using the Statistical Analysis System (SAS version 8.2).

7 RESULTS, DISCUSSIONS AND CONCLUSIONS

7.1 ANIMAL STUDY, EXPERIMENTAL

Paper I

Result

Effects of intradermal injection. Five of eight investigated common cosmetic products induced arthritis after intradermal injection. Mean day of onset was 13- 24 days after injection. The severity of arthritis was rather pronounced with arthritic score between 8 and 16 out of a maximum possible score of 16. The maximal disease severity occurred after 2-3 weeks of disease. Duration of the arthritis was from 5 to almost 9 weeks. Follow-up was at least 1 month (some 3 months). Intradermal injection with skin creams with high viscosity or with the vaginal gel did not induce any arthritis. None of 6 negative control animals that did not receive any injection developed any arthritis, nor did ten Lewis rats injected with a mineral oil.

Histopathology of arthritic joints verified arthritic reactions with synovitis at days 21, 25 and 33-post injection from animals injected intradermally with a baby oil, and histology of their lymph nodes showed increased number of cells and large round vesicles, probably disseminated oil droplets.

Serological analysis: Collagen II antibodies were detected in 4/10 animals previously injected with Margaret Astor skin cream containing collagen, oils, and liposomes.

Effects of percutaneous application. The baby oil that induced a very aggressive arthritis by intradermal injection was also assayed for arthritogenicity upon repeated percutaneous administration on abrased skin; it induced a mild and transient arthritis in 5 of 10 rats. The mean day of onset of arthritis was 13 days after treatment, and the maximum arthritic score was 4. Skin irritation tests with the baby oil gave no macroscopic changes of the skin, and microscopic evaluation performed on biopsies from the treated skin did not demonstrate any signs of inflammation.

Effects of per oral feeding. No arthritic reaction was seen during an observation period of 7 months.

Effects of intravaginal treatment. No arthritis recorded during a follow up of 3 months.

Effects of silicone implants. No arthritis observed during follow up of 6 months. Transient elevated fibrinogen levels were observed on days 6 and 17 after implantation of silicone.

Discussion

The aim of this study was to investigate if common commercially available cosmetic products that contain mineral oils or medicinal mineral oils can induce arthritis in the particularly arthritis-prone DA rat strain. The results clearly demonstrated that several of these products can induce arthritis after intradermal administration. One of them, baby oil, induced arthritis after percutaneous administration on the skin that had been moderately irritated by mild mechanical means. There was no measurable irritative reaction in the skin detectable by macroscopic or microscopic investigations. This agrees with previous observations that the effects of these nonimmunogenic adjuvants mainly occur in the regional lymph nodes in which the adjuvants cause activation of T lymphocytes, which have a capacity to subsequently induce arthritis. This also means, however, that such substances appear to be able to induce arthritis, at least after being applied to irritated skin, without giving rise to any types of local irritative reaction of the kind that are used to screen cosmetic products for potential irritation or other adverse effects.

Thus mineral oils in cosmetic products have retained their arthritogenic potential in the DA rats, even after being mixed with the many additional substances included in the various commercial products. In the present study only administration via the skin caused arthritis, whereas per oral or intravaginal applications did not give rise to any disease symptoms. We did not test the effects of oral or intravaginal adminstraion of adjuvant in conjunction with inflammation of the gut or vagina caused by some other agent, thereby paralleling the situation with abrased skin.

Silicone gel from breast implants has been suggested to be an adjuvant. However, silicone gel alone did not appear to be arthritogenic in our experiments, in agreement with other reports.

Conclusion

Mineral oils included in common commercially available products retain their adjuvant properties and are arthritogenic in the investigated arthritis-prone rat strain. A major final question was to what extent the findings in the particularly arthritis susceptible DA rat has any relevance for the situation in humans.

7.2 HUMAN STUDIES, OBSERVATIONAL

7.2.1 1. Register-based cohort study

Paper II

Result

In the cohort study male concrete and construction workers, farmers and male hair-dressers had increased cumulative incidence of hospital care due to RA compared with the normal population. When different occupational groups were combined according to various occupational exposures according to a job exposure matrix, employees in occupations with frequent exposure to organic solvents was observed to be at an increased risk of RA, whereas employees in occupations with frequent exposure to mineral oil were not. However, there was a small increase of the relative risk among male employees in several occupations that involve exposure to mineral oils such as toolmakers, machine-tool setters and operators and machinery and engine repairmen.

Discussion

In general there were rather small differences in the relative risk of RA in different exposure groups and different occupations. This could partly reflect a lack of strong occupational determinants of RA. However, it may also be due mainly to selection bias and exposure misclassification. In some occupations with an excess risk, patients with RA would probably have difficulties to remain employed due to the work load, and patients in light jobs had a greater chance to remain in their occupation. These tendencies would underestimate the risk of RA during the 1981-1983 in heavy occupations, and overestimate the risk in light occupations. Since chemical exposures tend to be more common in heavy, mostly blue-collar occupations, the estimates of the RR in the different exposure groups would also tend to be biased towards unity.

Since the RA diagnoses were only obtained during a 3 year period, and since many patients with RA probably never become in-patients, there must have been individuals with RA in our cohort who were classified as not suffering from this disease. This misclassification would not bias the relative risk if it was similar over occupational categories. However, there might be a tendency among those with heavy occupations to seek care more often than those with light occupations.

Conclusion

An increased risk of RA was determined in farmers and workers exposed to organic solvents despite probable extensive selection bias and exposure misclassification that are likely to have biased increased relative risks towards unity. A case-reference study of incident cases of RA and appropriate controls was suggested, in which hypotheses concerning the role of environmental risk factors for RA, including the risk confined by mineral oil exposure, could be further investigated.

7.2.2 2. Population-based case-control study

Papers III and IV

Papers III and IV are based on the same material. In total 1,480 cases and 2,038 controls were identified. Of these, 1,419 cases (1,012 women and 407 men) and 1,674 controls (1,188 women and 486 men) participated in the study, giving a participation rate of 96% for cases and 82% for controls.

Paper III

Result

Men reported a substantial occupational exposure to mineral oils while such exposure was uncommon among women. Thus the analysis was restricted to men. Among men, exposure to any mineral oil was associated with a 30% increased risk of developing RA (RR=1.3, 95% CI 1.0-1.7). When cases were subdivided into RF-positive RA and RF-negative RA, an increased risk was only observed for RF-positive RA (RR=1.4, 95% CI 1.0-2.0). When the cases were subdivided according to the presence of anti-CCP, an increased risk associated with exposure to any mineral oil was observed only for anti-CCP^+RA (RR=1.6, 95% CI=1.1-2.2). The increased risk remained after adjustment for smoking. Concerning exposure to different types of mineral oil, hydraulic oil was particularly associated with anti- CCP^+RA (RR=1.7, 95% CI 1.1-2.6). Analysis of the interaction between oil exposure and the presence of HLA-DR SE genes regarding the incidence of RA indicated that the increased risk associated with exposure to mineral oil was not related to the presence of SE genotypes.

Discussion

According to the results of our study, mineral oil appears to be associated with an increased risk of RF^+RA and anti-CCP^+RA.

Our study is a population-based case-control study using only incident cases of new diagnosed RA fulfilling the ACR criteria assessed by a specialist in rheumatology. A possible disadvantage with a case-control study with retrospective collection of exposure data is the risk for misclassification of exposure due to a recall bias that differs between cases and controls. Only incident subjects that received a diagnosis of RA for the first time were included to reduce the risk for recall bias, the mean duration between the estimated disease onset and inclusion in the study was ten months, and analyses of data of environmental exposures were only performed up to the index year. Bias due to change of habits, job or work exposure as a result of the disease was therefore probably limited. In order to investigate if possible misclassification of exposure to mineral oils differed between cases and controls an industrial hygienist performed an independent exposure classification without finding any signs of differential misclassification of exposure biasing the study.

Analysis of a possible interaction between the SE genes and exposure to mineral oils did not reveal any significant interaction. Although based on small numbers of observations, this suggests that mechanisms responsible for association between mineral oil exposure and RA may be different from those responsible for the association between smoking and RA, where a pronounced interaction between smoking and the HLA-DR SE genes was observed.

Conclusion

The study lends support to the hypothesis that exposure to mineral oil is associated with an increased risk of developing RF^{+}RA and anti- CCP^{+}RA, respectively. The finding is of particular interest since mineral oils can induce arthritis in rats.

Paper IV

Result

Most women were regular users of cosmetics such as body lotions and skin creams. As the number of women that reported no exposure at all was very low, different exposure categories regarding cosmetic use were compared with a low exposure group. Ten percent of female controls were "low users", whereas most of the male controls (74%) were "low users".

When analyzing the relationship between exposure to skin care products and risk of developing RA, no increased risk of developing RA in total was observed, neither for women nor for men; all observed relative risks were close to one. This was also the case when the analysis was restricted to different subgroups of RA, i.e. according to RF status and anti-CCP status, respectively.

Discussion

Results of this study provide no evidence that women or men exposed to common cosmetic ointments or body lotions have any increased risk of developing RA.

Our study is a population-based case-control study using only incident cases of new diagnosed RA fulfilling the ACR criteria assessed by a rheumatology specialist. The participation of almost all rheumatology units in the study area minimized the recruitment bias. Some cases in primary care may have been unidentified in our study. However, we know from population-based studies aimed at identifying RA cases directly in primary care that almost all cases of RA in our current Swedish system are indeed referred to rheumatology units. It is thus not likely that the relatively few unidentified cases would cause a substantial bias in our calculations. The response rate was high, with 96% for cases and 82% for controls, which limits risk for selection bias in this stage.

In our study information regarding cosmetic use was based on self-reported data about the frequency of cosmetics use on different parts of the body, as reported in a questionnaire.

A possible disadvantage with a case-control study with retrospective collection of exposure data is the risk for misclassification of exposure due to a recall bias that differs between cases and controls. Only subjects that received a diagnosis of RA for the first time were included to reduce the risk for recall bias, the mean duration between the estimated disease onset and inclusion in the study was ten months. In light of the observed results, bias due to non-differential misclassification of cosmetic use could in principle have led to diluted estimates of relative risks and hence have masked true positive associations. However, in our data, the observation of a tendency towards a decreased risk of RA among those with the highest exposure to cosmetics makes possible that non-differential misclassification of exposure has masked a true positive association between cosmetic use and RA unlikely. Nonetheless, there is a possibility that differential misclassification may have occurred. If

cases underreport true exposure to a higher extent than controls, this may indeed be the case. We have no reason to believe that this is the case but this a possibility that cannot be ruled out.
The usage of cosmetics among women is very common. Most women use these products in any form more or less regularly. The proportion of unexposed was consequently very low, only 0.89% of female cases and 0.59% of the female controls, which hampered the use of totally unexposed as reference category. Among men, on the contrary, 15.9% were non-users. We performed analysis comparing men exposed to cosmetics to various degrees with totally unexposed men. Still no increased risks associated with cosmetic use were observed.
To increase the interpretability of negative studies, as in the current one, it is of interest to know the power of the study, or what magnitude of risk difference between the exposed and unexposed the study reasonably would detect. The size of the current study was sufficient (power 0.80) to detect a risk increase in the order of 20-30% (for women and men together).

In paper III we reported an association between occupational exposure to mineral oil and $RF^{+}RA$ and anti $CCP^{+}RA$, respectively, among men. The male occupational exposure to mineral oil probably comprises both larger quantities and higher concentration of mineral oil than is the case during use of cosmetics, and in addition, occupational exposure to mineral oil often may often occur with simultaneous damage of the skin.

Thus in summary, even though occupational exposure to mineral oils seem to be associated with an increased risk of RA, the use of skin care products with content as that during the 1990s does not seem to be a risk factor for RA in the population in general. However, the products and their content were unknown and there may be great differences in arthritogenicity between different products depending on type of oil and mineral oil content, and the individual genetic disposition.

Conclusion

This study does not support the hypothesis that usage of common cosmetics as body lotions, skin creams, and ointments, often containing mineral oil, increase the risk for RA in the population in general. We cannot exclude, however, that these cosmetics can contribute to arthritis in individuals carrying certain genotypes or simultaneously being exposed to other arthritis inducing environmental agents.

8 GENERAL DISCUSSION AND CONCLUDING REMARKS

Arthritis can be induced in many ways, as a result of different arthritogenic bacteria, viruses, mineral oil and squalene among others. RA is a symptom complex, probable a disease of multifactorial origin For more than 50 years mineral oil has been known to be an efficient immunological adjuvant, and an important constituent in the most used immunological adjuvants, FCA and FIA, in inducing autoimmunity and arthritis in animals.

The results of this thesis (Table 4) disclose that in human mineral oil exposure also seems to be associated with an increased risk of developing RA, especially RF^{+}RA and anti- CCP^{+}RA. The animal experimental study revealed that common cosmetic products containing mineral oil induced arthritis in the DA rat. However, the epidemiological case-control study could not reveal any increased risk of developing RA by cosmetic users. Conversely, which products they had used was unknown. In addition, in the rat experiments we had chosen common commercial products with a general high degree of mineral oil. Besides, the most evident animal arthritis was induced by intradermal injection. The experimental arthritis induced by percutaneous administration on abrased skin was mild and of short duration, the corresponding human transient arthritis would likely pass by without any registration as early arthritis or RA. Thus we cannot exclude that a few individuals exposed to both skin trauma and certain mineral-oil containing cosmetics may develop arthritis, but this is unlikely to be a common event.

9 POINTS OF PERSPECTIVE – FUTURE

The EIRA study implies possibilities to further study the effect of environmental factors in combination with genetic inheritance, to try to analyze the role of adjuvant exposure by vaccination, possibilities to further study the role of mineral oil as an adjuvant in connection with other immunological adjuvant such as different infections, and together with solvents, meaning increased possibility to close contact with the immune system, and giving the proper signals to the proper cells. Finally for the future, I have a hope, that when it is unnecessary, do not stimulate the immune system, and protect us from harmful exposure.

10 SAMMANFATTNING (SUMMARY IN SWEDISH)

Omgivningsfaktorer bidrar sannolikt till uppkomst av ledgångsinflammation, reumatoid artrit (RA).

Mineralolja är en effektiv adjuvant (förstärkare) av den immunologiska reaktionen. Mineralolja används därför vid immunisering av djur och i djurmodeller av olika autoimmuna sjukdomar såsom RA.

Ämnet för denna avhandling är att i djurförsök och genom human epidemiologiska studier belysa mineraloljans roll som immunologisk förstärkare vid RA.

Djurförsök med DA råtta visade att fem av åtta testade vanliga kommersiella kosmetiska produkter, som innehöll mineralolja, gav ledinflammation vid injektion i huden. En barnolja gav dessutom en övergående, lätt ledinflammation, då den ströks på lätt irriterad, rakad hud.

Epidemiologiska studier gjordes för att undersöka yrkesmässig exponering för mineralolja och risk att utveckla RA. Först företogs en registerbaserad kohort studie, där den kumulativa incidensen av RA jämfördes mellan olika yrkesgrupper. Studiepopulationen omfattade personer födda 1905-1945, som 1980 var bosatta i något av 13 svenska landsting och uppgett samma yrke vid folkräkning 1960 och 1970. Studiepopulationen följdes rörande vård för RA under 1981-1983 i slutenvårdsregistret. En liten ökad risk för RA observerades för manliga lantarbetare, tapetserare, lackerare, betongarbetare, och frisörer, liksom för verktygsmakare, maskinister och maskinreparatörer, yrken förenliga med mineraloljeexponering, även om den kemiska exponeringen enligt en jobb-exponerings matris inte visade någon ökad risk för dem, som exponerats för mineralolja, utan endast ökad risk för dem som exponerats för lösningsmedel. Därefter genomfördes en populationsbaserad fall-kontroll studie omfattande 1419 nydebuterade RA fall och 1674 kontroller. Män som varit yrkesmässigt exponerade för mineralolja visade ökad risk att utveckla RA (relativ risk (RR) = 1.3, 95% konfidensintervall (KI) = 1.0-1.7). Mineraloljeexponering var speciellt förenad med ökad risk att få reumafaktor positiv RA (RF^+RA) (RR =1.4, 95% KI =1.0-2.0) liksom anticitrullin positiv RA (anti-CCP^+RA) (RR =1.6, 95% KI=1.1-2.2). Högsta risk att få anti-CCP^+RA observerades vid exponering för hydraulolja (RR=1.7, 95% KI=1.1-2.6). Antalet kvinnor, som var yrkesmässigt exponerade för mineraloljor, var för lågt för att tillåta meningsfulla analyser.

Kosmetikaanvändning i form hudvårdsprodukter såsom kroppslotioner och krämer mm, som också undersöktes i fall-kontroll studien, tycktes inte vara förenad med en ökad risk för RA. Vilka preparat, som hade använts, var dock inte känt

Slutsats

Det är sedan tidigare känt att mineralolja är en effektiv immunologisk adjuvant i djurförsök och används för att framställa djurmodeller av RA liksom flertal andra autoimmuna sjukdomar. I föreliggande avhandling observerades att även vanligt förekommande kosmetiska preparat som innehåller mineralolja gav ledinflammation på DA råtta. Den epidemiologiska studien tyder på att yrkesmässig exponering för mineralolja hos män innebär ökad risk att få RA. Någon ökad risk för RA vid kosmetikaanvändning kunde inte konstateras.

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11 TABLES

Table 1 Different chemical adjuvants

Adjuvant substance		**Vaccination against**	**Selected reference**
Aluminum salts	$Al(SO_4)_2$ $AlPO_4$ $Al(OH)_3$	Tetanus, diphtheria, whooping cough, hepatitis, tick born encephalitis (TBE)	(155)
Calcium phosphate		(Diphtheria, tetanus)	(155)
Endotoxins			(156)
Bordetella (endotoxin)			(157)
Mycobacterium (Wax D)			(158)
Retinol			(159)
Silica			(160)
Beryllium			(161)
Saponin	(amphiphatic, surface active, particularly effective adjuvant for antigens in cell membranes -possible interaction with cholesterol in a cell membrane)		(162)
Oil emulsions	Mineral oil, FCA, FIA (depot adjuvant)	Veterinary medicine, previously in influenza vaccine given to American military, but now not admitted in USA	(135)
	Animal oils – squalene (MF 59)	Influenza vaccine	(163)
	Vegetable oils – peanut, sesame oil, retinol (vitamin A), carrageenan		(135)
Synthetic adjuvants	MDP(murmyl dipeptide, derived from mycobacteria - depot adjuvant), liposomes, RNA complex, anionic polymers, polyacryl- and lipopeptides, lipopolysackarides (LPS, Lipid A), immune stimulating complex (ISCOM)		(135)
Adjuvant molecules	Peptidiglycan- MDP (M. tuberculosis) Polysaccharide – β-glucan (S. cerevisiae) Glykolipid – LPS (E.coli), TDM (M. tuberculosis) Lipid $-C_{30}H_{50}$ (squalene) $C_{38}H_{80}NBr$ (DDA) $C_{43}H_{52}N_20_2$ (avridine) $C_{19}H_{40}$ (pristine) $C_{16}H_{34}$ (n-hexadecane)		(143)

Table 2 Experimental autoimmune diseases induced with FCA

Human disease	Experimental (Exp) disease induced in animal	Animals	Antigen	Some selected references
Ankylosing spondylitis	Exp. autoimmune spondyl(odisc)itis	Rat	Nucleus pulposus	(164) (165)
Aspermatogensis	Exp. aspermatogensis	Guinea pig	Spermatozoa	(166)
Diabetes mellitus	Exp. diabetes mellitus	Rat	Endocrine pancreas extract	(167)
Glomerulonephritis	Exp. glomerulonephritis	Rat	Kidney suspension	(168)
Guillain-Barré syndrome	Exp. allergic neuritis	Lewis rat	Spinal cord extract	(169)
Hashimoto's thyroiditis	Exp. autoimmune thyroiditis	Rat	Thyroid extract	(170) (171)
Liver necrosis	Liver necrosis	Guinea pig, hamster	Whole liver	(172)
Mb Addison	Exp. adrenalitis	Guinea pigs	Adrenal antigen	(173)
Multiple sclerosis (MS)	Exp. autoimmune encephalomyelitis (EAE)	Lewis rat	Spinal cord extract	(174)
Myastenia gravis (MG)	Exp. MG	Rat Guinea pig	Acetylcholine receptor	(175)
Myocarditis	Experimental autoimmune myocarditis	Mice	Cardiac myosin	(171)
Nephritis	Exp. interstitial nephritis	Mice	Renal tubular basement membrane	(176)
Neuritis	Allergic neuritis	Rabbit	Peripheral nervous tissue	(177)
Orchitis	Experimental allergic orchitis	Lewis rat	Testicular homogenate	(178)
Pernicious anaemia	Exp. pernicious anaemia	Mice	Parietal cells	(179)
Poly-dematomyositis	Exp. myositis in rats	Rat	Muscle extract	(180)
Postvaccinal and postinfecious encephalomyelitis	EAE	Guinea pig	Brain or spinal cord extract	(181)
RA	Erosive polyarthritis	Rat, mice, rabbit, monkey	Muscle, collagen II, ovalbumin	(139), (182), (183), (184)
RA	Polyarthritis	Horse	-	(185)
Sjögrens (sicca) syndrome	Exp. sialoadenitis	Guinea pig, Lewis rat	Salivary gland extract	(186), (187)
SLE	Ds DNA-antibodies	Mice	Purified double stranded DNA from E. coli	(188)
Uveitis	Exp. autoimmune uveitis	Rat	Nervous tissues	(189)
Vasculitis	Exp. vasculitis	Rat	Vascular endothelium	(190)

Table 3 RA animal model diseases

Induced with live microbial agents	Substance	Author	Reference
	Erysipelothrix insidiosa	Sikes et al	(191)
	Mycoplasma hyorhinis	Decker & Barden	(192)
	Mycoplasma arthritdis	Washburn et al	(193)
	Salmonella enteritidis	Volkman & Collins	(194)
	Herpes simplex virus	Bacon et al	(195)
Adjuvant arthritis			
+FCA	Mycobact. tuberculosis	Pearson CM	(139)
+ FCA	Corynebact. rubrum	Paronetto	(196)
+FCA	Synthetic adjuvant	Chang Y, Pearson C, Abe C	(197)
FIA			(198)
Pristane (C19 H40)			(199)
Antigen induced arthritis			
+ FCA	Fibrin	Dumonde & Glynn	(200)
+ FCA	Methylated bovine albumin	Brackertz et al	(201)
+ FCA (for collagen immunizations)	Collagen II	Trentham et al	(202)

Table 4 General results and discussion

Nr	Paper	Year	Study	Results and conclusions	Objections	Previous studies
I	Lundberg I, Alfredsson L, Plato N, Sverdrup B. et al. Occupation, occupational exposure to chemicals and rheumatological disease.	1994	Human, observational Register-based cohort study.	There was a small increased risk among male employees in several occupations that involve exposure to mineral oils such as toolmakers, machine-tool setters and operators and machinery and engine repairmen. However, among workers exposed to oils according to the JEM no increased risk of RA was observed. An increased risk of RA was found in farmers and workers exposed to organic solvents.	Selection bias and exposure misclassification	[81, 91]
II	Sverdrup B, Klareskog L, Kleinau S. Common commercial cosmetic products induce arthritis in the DA rat. .	1998	Experimental animal, rat study	Mineral oils included in common commercially available products retain their adjuvant properties and are arthritogenic in the investigated arthritis-prone rat strain	DA rat very sensitive	[142]
III	Sverdrup B, Källberg H et al.: Association between occupational exposure to mineral oil and rheumatoid arthritis.	2005	Human Case-control	Men occupationally exposed to mineral oil appear to have an increased risk to develop RF^{+} RA and anti-CCP^{+} RA.		[81]
IV	Sverdrup B, Källberg H et al.: Usage of skin care products and rheumatoid arthritis. Manuscript.	2007	Human Case-control	This study does not support the hypothesis that usage of common cosmetics such as body lotions, skin creams, and ointments, often containing mineral oil, increase the risk for RA in the population in general	Products and content were unknown. Differences in arthritogenicity between different products, their type of oil and oil content, and the individual genetic disposition. We cannot exclude, however, that these cosmetics can contribute to arthritis in individuals carrying certain genotypes or simultaneously being exposed to other arthritis inducing environmental agents.	[81]

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