# 1 Introduction

## 1.1 Autoimmune diseases

Despite decades of intensive study, the primary underlying causes of most autoimmune diseases (AIDs) remain uncertain. The etiopathogenesis of, for example, multiple sclerosis (MS) or rheumatoid arthritis (RA) is still unsolved. Nevertheless, different genetic, environmental, and infectious factors contributing to the development of AIDs have been described. Conversely, additional findings imply that these factors are not the sole cause of these diseases. Thus, a multifactorial origin for at least a share of the more than eighty autoimmune diseases described so far has to be assumed (see addendum for a compilation of AIDs; 5.1).

Due to clinical pictures of retrovirus infection seen in animals, and especially since the discovery of human pathogenic retroviruses in the early 1980s, members of this virus family were suspected to play a role in the etiopathogenesis of slowly progressing diseases. However, the known human pathogenic retroviruses could not be linked to AID, and final proof of other retroviruses could not yet be obtained. If yet undiscovered or zoonotically transmitted retroviruses are involved, the question arises how something unknown can be reliably detected? In the case of endogenous viruses, aberrant expression may have detrimental effects, but what is the difference between ‘regular’ expression (if there is any at all) and aberrant expression? And if retroviruses do not play a role in etiopathogenesis, can absence be proven?

### 1.1.1 Definition of autoimmune disease

Autoimmune diseases represent the largest subgroup of slowly progressing diseases. In contrast to acute diseases, clinical pictures are not observed in close time proximity to an infection. Disorders of manifold clinical outcomes are compiled under the umbrella of slowly progressing diseases: respiratory, rheumatoid, immunosuppressive, genitourinary, cardiac, gastrointestinal, and skin diseases as well as syndromes like Chronic Fatigue Syndrome (CFS), Fibromyalgia Syndrome (FMS), and Gulf War Illness (GWI).

In principle, AID could be considered the result of failed homeostasis in the immune system, leading to the activation of autoreactive T cells and/or antibodies which can cause tissue damaging lesions or metabolic dysfunctions. Many chronic inflammatory diseases have been ascribed to autoimmunity. Organ-specific diseases may attack single cell types (e.g. beta cells in pancreatic islet in insulin dependent diabetes mellitus, IDDM), systemic AIDs may affect single organ systems (the central nervous system, CNS, and the white matter in MS), or may devastate many tissues (e.g. systemic lupus erythematosus, SLE). Besides organ-specific and systemic AIDs, mixed or transitional diseases have been described as well (e.g. Goodpasture's syndrome). The variety of tissues affected in systemic AIDs may increase with time (Whitton and Fujinami, 1999). Immune responses

directed against agents which induce persistent infections, and not against self-antigens, can also cause tissue damage. A strict definition of autoimmunity would exclude such diseases, because T cells or antibodies specific for self-antigens are not responsible for tissue damage. However, in practice in can be difficult to make a clear distinction.

### 1.1.2 Epidemiology of autoimmune diseases

Autoimmune diseases affect 3%-7% of the human population (Marrack et al., 2001, Rouse and Deshpande, 2002, Wraith et al., 2003, Jacobson et al., 1997, Kuby, 1997). Prevalence rates range from less than 5 per 100,000 (e.g. chronic active hepatitis, uveitis) to more than 500 per 100,000 (Graves' disease, rheumatoid arthritis, thyroiditis). The diseases with the highest prevalence are Graves'/hyperthyroidism, IDDM, pernicious anemia, rheumatoid arthritis (RA), thyroiditis, and vitiligo, comprising an estimated 93% of the total number estimated. Glomerulonephritis, MS, and SLE affect about 4% of the overall afflicted. The presence of other AIDs is rare (Jacobson et al., 1997). While the prevalence of rheumatoid arthritis has been stable or has even declined over the last 30 years (Uhlig and Kvien, 2004) the prevalence of other AIDs, e.g. IDDM has been rising (Jacobson et al., 1997, Cooper and Stroehla, 2003, Steen et al., 1997, Uramoto et al., 1999). These findings could be due to higher incidence rates and/or improved diagnostic options and prolonged survival time (fig. 1).

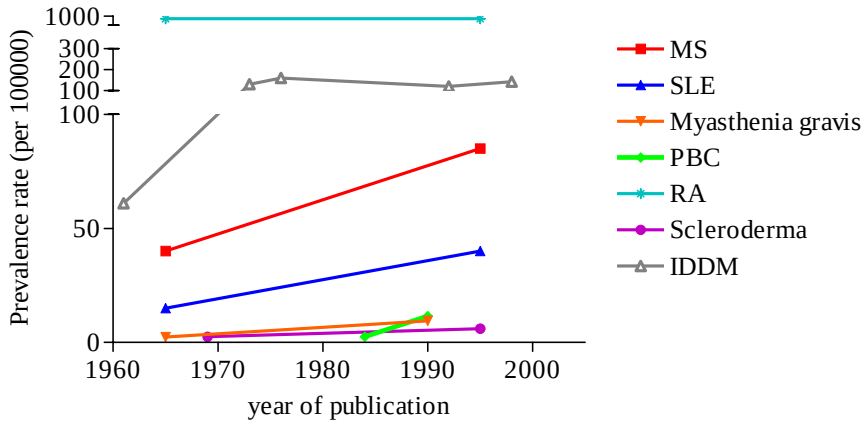


**Fig. 1: Prevalence rates of AIDs by year of publication.** The prevalence rates are given per 100,000 inhabitants for all diseases. MS: multiple sclerosis, SLE: systemic lupus erythematosus, PBC: primary biliary cirrhosis, RA: rheumatoid arthritis, IDDM: Insulin dependent diabetes mellitus (USA, age <18; data according to Jacobson et al., 1997, Sultz et al., 1972, National Center for Health Statistics 1977a, 1977b, 1994, Harris et al., 1998).

Autoimmune diseases are generally thought of as being relatively rare. As a group, however, they cause significant severe and chronic morbidity and disability (e.g. RA, SLE, and rheumatic fever) as well as being an important cause of mortality and shortened life expectancy (e.g. scleroderma and myocarditis; Riise et al., 2001). AIDs are among the ten leading causes of death among young and middle-aged women (ages <65 years) in the United States (Walsh and Rau, 2000, Jacobson et al., 1997) and they are the third most common group of diseases in Germany (following cardiovascular diseases and cancer, www.vdgh.de). Besides the loss of quality of life for the afflicted, many of these diseases

occur among young and middle-aged adults. Thus, they also are significant contributors to productive years lost in this part of the population leading to significant indirect costs. Since AIDs are chronic disorders, lifelong care and monitoring is required, resulting in significant direct costs for medical care utilization.

### 1.1.3 Gender-specific distribution of autoimmune diseases

Almost all autoimmune diseases disproportionally affect women (about 75% to 85%; Cooper and Stroehla, 2003, Molina and Ehrenfeld, 2003, Jacobson et al., 1997; Kuby, 1997). The immune effects of sex steroids can be observed particularly during pregnancy. Then, many patients with established AIDs experience an improvement. This applies in particular to thyroid AID and RA. An explanation may involve the increased level of immune tolerance induced by the pregnant state (see addendum for examples of gender related distribution of selected AIDs; 5.2).

### 1.1.4 Onset of autoimmune diseases

There are notable differences in the age distribution among autoimmune diseases (Cooper and Stroehla, 2003). Although most diseases occur at any age, there are clear peaks of onset. Some diseases primarily occur in childhood and adolescence (type 1 diabetes), in the mid-adult years (myasthenia gravis, MS), or among older adults (RA, primary systemic vasculitis). Some organ-specific (e.g. thyroid and adrenal) and systemic AIDs (RA) have a predilection for the older aged and/or female gender. The effect of aging on the incidence of certain AIDs is generally ascribed to the decline in immune function in old age, so-called ‘immunosenescence’ (Boren and Gershwin, 2004). However, a broad range of age at onset is seen in many diseases; for example, the onset of MS is usually seen in early adulthood, but in about five percent of the overall MS population the onset of disease is before the age of 16 (early onset MS, EOMS; Pohl et al., 2004; see addendum for examples of age at onset of selected autoimmune diseases; 5.3).

### 1.1.5 Etiology of autoimmune diseases

Many slowly progressing diseases have been studied for decades without the etiology being clarified. However, findings suggest a multifactorial origin for most autoimmune diseases; the factors involved could include genetic predisposition – on familial as well as ethnical basis -, environmental factors (climate, nutrition, exposition to chemicals etc.), infectious agents, gender and age.

#### 1.1.5.1 Genetic risk factors for autoimmune diseases

Host genes affect the susceptibility to autoimmunity at three levels. First, some genes affect the overall reactivity of the immune system and thus can predispose the individual to a variety of different types of AID. Second, genes which affect T cell recognition of peptides direct this altered immunoreactivity to particular antigens or tissues. Third, genes can act on the ability of target tissues to modulate the immune response. These last two

sets of genes determine which antigens will be the targets of autoimmunity and hence which organs will be attacked and what damage will occur. In addition, signals from the environment can influence the development of autoimmunity at the same three levels, by affecting the overall reactivity of the immune system, antigen-specific response and the state of the potential target tissue (Marrack et al., 2001). A polygenic predisposition with the additional requirement of exposure to environmental or external triggers is probably responsible for the onset and perpetuation of most AIDs (Becker et al., 1998, Luppi et al., 1995, Miller, 1995). The highest risk factors for many AIDs are those associated with the HLA loci on human chromosome 6 (Shamim and Miller, 2000). Non-HLA loci implicated as risk factors for autoimmunity include regions encoding immunoglobulins, cytokines, their receptors, and T cell receptors (Carson, 1992, Robinson and Kindt, 1992). Genes that act directly on the immune system are frequently not disease specific. The clustering of different AIDs in the same families occurs more often than predicted by the prevalence of each disease in the population. For example, it is not uncommon for patients with SLE or autoimmune myositis (polymyositis) to report family members with other systemic rheumatic diseases or even organ-specific AIDs (Shamim and Miller, 2000, Lin et al., 1998, Midgard et al., 1996, Strom et al., 1994). Another cluster involves type 1 diabetes, autoimmune thyroiditis, Addison's disease, autoimmune polyendocrine syndromes, vitiligo and celiac disease in various combinations (Verge and Eisenbarth, 1992). Thus, the genetic risk factors for autoimmunity consist of two forms: those that are common for many AIDs, and those that are specific for a given elemental disorder.

#### 1.1.5.1.1 Genetic predisposition: familial risk factors for autoimmune diseases

Many studies have suggested a genetic role in the pathogenesis of AID (Shamim and Miller, 2000, Morahan and Morel, 2002, Miller 1995; Becker et al., 1998). Twin studies have demonstrated that if one monozygotic twin develops an autoimmune disease, the other twin has roughly 25% to 60% concordance, depending on the autoimmune disease in question (Fujinami, 2001). Concordance rates of approximately 25% in monozygotic twins have been described for most common autoimmune disorders, such as RA, SLE; IDDM and MS (Perl, 2003).

#### 1.1.5.1.2 Genetic predisposition: ethnic risk factors for autoimmune diseases

Differences regarding the risk of specific autoimmune diseases among countries or among ethnic groups living in the same area have been reported. The pattern is not consistent across autoimmune diseases, however, as specific ethnic groups may be at higher risk for some diseases but lower risk for others. For example, RA and scleroderma affect a higher percentage of residents in some Native American communities than in the general US population, while rates for RA are similar among Whites, Blacks and Hispanics (Cooper and Stroehla, 2003).

#### 1.1.5.2 Environmental factors in autoimmune disease

The discordance rate observed in twin studies (appr. 40% to 75%) emphasizes the importance of exogenous factors, although genetic susceptibility might be a prerequisite for onset of autoimmune disease. Environmental factors could comprise dietary intake, smoking, toxic agents, drugs, heavy metals, stress and many more variables such as sunlight or climate (Riise et al., 2003, Riise et al., 2002, Mayes et al., 2003, Ghaussy et al., 2003, Ghardirian et al., 1998, Koziol and Feng, 2004, Willer et al., 2005).

#### 1.1.5.3 Infectious agents in autoimmune disease

Microbial agents infecting a host can either be cleared by the immune response or persist due to immune escape. Both types of infection can result in autoimmune or autoimmune-like diseases. Persistent viral infection can be associated with detrimental consequences remote from the initial infection. Examples of this include chronic hepatitis with hepatitis B (HBV) and hepatitis C (HCV) as well as the neurological diseases subacute sclerosing panencephalitis (SSPE) and progressive multifocal leucoencephalopathy (PML), associated with the persistence of measles and polyomavirus, respectively (Portis, 2002). However, viruses can trigger AIDs and be subsequently cleared, i.e. at onset of disease virus is not detectable (molecular mimicry, 1.1.6.1). Pathologies are either a consequence of direct cytopathic effect of the virus infection or secondary to inflammatory responses it engenders (see addendum for a compilation of proposed AIDs for which viral infections have been suggested; 5.4).

##### 1.1.5.3.1 Retroviruses and autoimmune disease

A role of retroviruses in the etiopathogenesis of autoimmune diseases has been suspected for a long time but has not yet been proven. However, multiple findings support this hypothesis. Antibodies reacting with human T cell leukemia virus (HTLV-1) and human immunodeficiency virus (HIV-1) antigens have been described in some patients with autoimmune diseases, especially RA, SLE and Sjogren's syndrome (Brookes et al., 1992, Maul et al., 1989, Talal et al., 1990a, Talal et al., 1990b, Ziegler et al., 1989, Zucker-Franklin et al., 2002). Endogenous retroviruses (ERVs, 1.3.6.1) are well recognized as causes of a range of disease processes in naturally and experimentally infected animals. Infection with ERVs in animals can not only result in neoplasia, fetal malformations, and encephalitis, but also in autoimmunity (Yolken et al., 2000). Furthermore, several autoimmune diseases have been studied in animal models with the respective disease triggered by retrovirus infection. The caprine arthritis encephalitis virus (CAEV) causes chronic arthritis in goats (Crawford et al., 1980), the murine leukemia virus (MLV) is involved in the onset of an SLE-like disease in New Zealand mouse strains (Yoshiki et al., 1974) and HTLV-1 transgenic mice developed neurofibromas, or showed Sjogren's syndrome-like symptoms (Green et al., 1989).

Aberrant expression of human endogenous retroviruses (HERVs) has been associated with a number of chronic human diseases, including MS, diabetes and autoimmune arthritis (Conrad et al., 1997, Perron et al., 1997, Nakagawa et al., 1997, Murphy et al., 1998, Lower et al., 1998, Lan et al., 1998). Basically, investigations using PCR results precluded HTLV and HIV infection as cause for rheumatoid diseases. However, the observed cross-reactivity with HIV and HTLV antibodies indicates involvement of retroviral agents to some extent.

## 1.1.6 Potential mechanisms for induction of autoimmunity

Different mechanisms for activation of autoreactive T and B cells by infectious agents have been proposed. Infectious agents such as exogenous retroviruses (1.3.7) could induce AID directly by appearance of a neoantigen, resulting in molecular mimicry, generation of new epitopes (loss of tolerance) and induction of superantigens. Furthermore, endogenous retroviruses could induce AID indirectly.

### 1.1.6.1 Molecular mimicry

Molecular mimicry is defined as activation of autoreactive T cells by microbial peptides that have sufficient structural similarity to self-peptides. Either the molecules' linear amino acid sequences or their conformational structures may be shared, even though their origins are as separate as, for example, a virus and a normal host-self determinant. Roughly 5% of monoclonal antibodies made against 15 different viruses cross-reacted also with 'host-self determinants' when over 800 independent monoclonal antibodies were studied (Oldstone, 1998). Several viral proteins were uncovered that showed significant homology with the encephalitogenic site of myelin basic protein (MBP), including fits between the MBP and nucleo-protein of the haemagglutinin of influenza virus, core protein of adenovirus, EC-LF2 protein of Epstein-Barr virus, and HBV polymerase, as well as others (see addendum for examples of possible cross-reactive immune responses; 5.5). These results suggest that molecular mimicry could lead to autoimmune disease. Furthermore, the infection of cells can induce cellular injury releasing self antigens, which generate immune responses against self-antigens.

### 1.1.6.2 Superantigens

Superantigens are proteins produced by bacteria and viruses that cause massive stimulation of immune cells, also resulting in host damage. Exogenous superantigens are soluble proteins secreted by bacteria, including a variety of exotoxins secreted by gram-positive bacteria. Endogenous superantigens are cell-membrane proteins encoded by certain viruses that infect mammalian cells. Superantigens bind to residues in the variable (V) domain of the T cell receptor (TCR) and to residues in class II MHC molecules outside of the antigen binding cleft. In this way a superantigen can cross-link a T cell to a class II MHC molecule even when the TCR does not recognize the bound antigenic peptide, leading to activation

of the T cell. A superantigen can thus activate all T cells expressing the V domain to which that superantigen binds. The mouse mammary tumor virus (MMTV) has an additional gene called 'sag' for superantigen (Choi et al., 1992, Choi et al., 1991). An MHC class II dependent superantigen is also encoded within the *env*-region of HERV-K (IDDMK$_{1,2}$22). RNA sequences of this MMTV-related human ERV have been detected in the plasma of IDDM patients (Conrad et al., 1997).

#### 1.1.6.3 Bystander activation

Autoimmunity may be induced by virus infection in the absence of shared antigens. Several potential mechanisms are conceivable for this bystander activation (Wucherpfennig, 2001, Whitton and Fujinami, 1999). An infection could alter a host protein's structure, rendering it antigenic; virus-mediated cell destruction may lead to the release of large quantities of normally sequestered host protein; a virus might cause expression of a normally quiescent self gene, and/or virus infections often result in high local concentrations of cytokines, which may activate local resting responses. Viruses may also infect and subvert cells of the immune system, for example, Epstein-Barr virus (EBV) infection of B cells leads to polyclonal B cell activation, which may explain the frequent appearance of autoantibodies and the apparent association between EBV and AID.

#### 1.1.6.4 Activation of lymphocytes by lymphotropic viruses

Viral infection of lymphocytes, such as infection of B cells with HCV, can result in a lymphoproliferative disease. The B cell proliferation leads to enhanced antibody production and the formation of circulating immune complexes. A persistent virus infection of lymphocytes can result in an immune mediated disease (Wucherpfennig, 2001).

#### 1.1.6.5 Induction of autoimmune disease by endogenous retroviruses

Quantitatively or structurally aberrant expression of endogenous retrovirus (ERV) sequences could induce autoimmunity. Aberrant ERV expression could activate or suppress genes which are involved in immune function. Also cis- and transregulation of genes could cause detrimental effects for the host.

## 1.2 Specific autoimmune diseases

### 1.2.1 Multiple sclerosis

Multiple sclerosis (MS) is the prototype inflammatory autoimmune disorder of the central nervous system and, with a lifetime risk of one in 400, supposedly the most common cause of disability in young adults (Compston and Coles, 2002). The mean total lifetime cost per patient of MS has been estimated to be $2.5 million in 1994 (Patwardhan et al., 2005). Although indication of MS dates to the late 14$^{th}$ century (Medaer, 1979) the first documented case of MS was described by Augustus d'Este in the 1820's who described the

clinical picture of the disease several decades before the definite description by Charcot in 1868 (McDonald, 2002).

### 1.2.1.1 Epidemiology of multiple sclerosis

#### 1.2.1.1.1 Prevalence of multiple sclerosis

MS is the third leading cause for neurological disability after trauma and arthritic disease. It is estimated that 1 to 2.5 million people suffer from MS worldwide (fig. 2; Dyment et al., 2004, Rudick et al., 1997, Compston and Coles, 2002, Achiron and Mandel, 2004, www.msif.org, 2004).

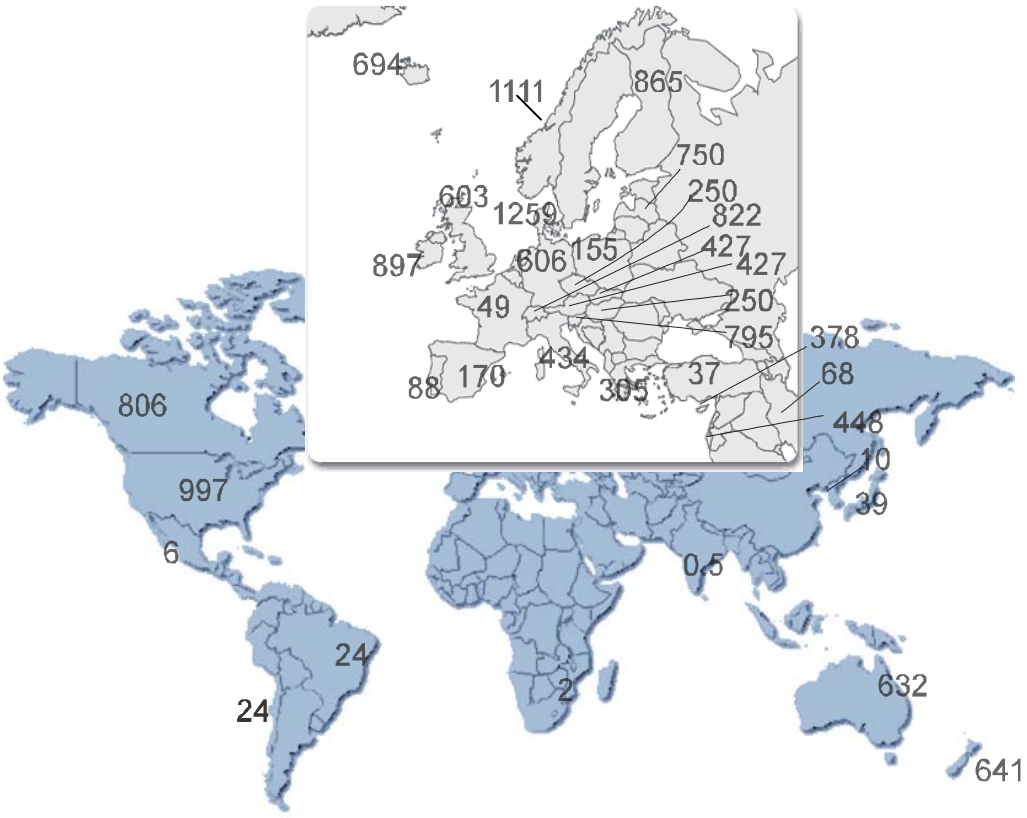


**Fig. 2: MS prevalence.** MS cases per million inhabitants (data according to: www.msif.org, 2004, www.auswaertigesamt.de, 2004).

#### 1.2.1.1.2 Gender related characteristics of multiple sclerosis

MS is more commonly observed in females than in males, occurring approximately 50% more frequently in women than in men with ratios of 2:1 to 3:2 (Hellings et al., 2004, Achiron and Mandel, 2004, Rudick et al., 1997, Compston and Coles, 2002, www.msif.org, 2004). In MS, the female to male ratio seems to increase with pre-puberty onset and to vary geographically (Dyment et al., 2004).

#### 1.2.1.1.3 Onset of multiple sclerosis

Age at onset varies depending on the study; it is mainly described between ages 20 and 40 (Achiron and Mandel, 2004, Compston and Coles, 2002, www.msif.org, 2004). About 2% of patients with MS present before age 10, and 5% before age 16 (early onset MS, EOMS; Boiko et al., 2002, Duquette et al., 1987, Ghezzi et al., 1997, Liguori et al., 2000, Simone et al., 2002, Sindern et al., 1992). EOMS was not described in earlier MS studies, thus, this form of MS might represent a recent change in onset and progression of the disease. For example, a progressive decrease of the mean age of onset from 1976 (41 years old) to 2001 (22 years old) was described for a Sardinian cohort (Cocco et al., 2004).

### 1.2.1.2 Pathology of multiple sclerosis

Multiple sclerosis is an immune-mediated disease of the CNS that is characterized by inflammation, multiple sclerotic lesions or plaques found in the white matter of the CNS, loss of myelin (demyelination), and axon loss. These effects can result as a consequence of direct damage to myelin by inflammatory cells or indirectly because of the environment produced by inflammation. Demyelination is accompanied by a disruption in the ability of the nerves to conduct electrical impulses to and from the brain and this produces the various symptoms of MS, ranging from motor, sensory, coordination, strength, visual and/or cognitive impairment, tremor, nystagmus, paralysis, and disturbances in speech, swallowing, vision, sexuality, as well as urinary or bowel dysfunction and symptoms of fatigue (Achiron and Mandel, 2004, Lutton et al., 2004, www.msif.org, 2004). Breakdown of the blood-brain barrier is a crucial event in MS pathogenesis (Lutton et al., 2004). Clinical manifestations and the degree of disability are extremely variable. They may vary over time and can change in severity and duration, even in the same person. MS is grouped into five clinical categories which are overlapping:

1. Relapsing-remitting (RR): Episodes of acute worsening with recovery and a stable course between relapses. In this form of MS there are unpredictable relapses (exacerbations, attacks) during which new symptoms appear or existing symptoms become more severe. This can last for varying periods (days or months) and there is partial or total remission (recovery). The disease may be inactive for months or years; frequency: 25% to 85% (Achiron and Mandel, 2004, Compston and Coles, 2002, www.msif.org, 2004).
2. Primary progressive (PP): Gradual, nearly continuous neurological deterioration from the onset of symptoms. This form of MS is characterized by a lack of distinct attacks, but with slow onset and steadily worsening symptoms. There is an accumulation of deficits and disability which may level off at some point or continue over months and years. Patients with PP tend to be older at onset (40 to 60 years) and commonly have a progressive myelopathy; frequency: 10% to 20% (Achiron and Mandel, 2004, Compston and Coles, 2002, www.msif.org, 2004, Rudick et al., 1997, Pender and Wolfe, 2002).

3. Secondary progressive: Gradual neurological deterioration with or without superimposed acute relapses in patients who previously had relapsing-remitting MS; frequency: 40% to 50% (Achiron and Mandel, 2004, Rudick et al., 1997, www.msif.org, 2004).
4. Benign: After one or two attacks with complete recovery, this form of MS does not worsen with time and there is no permanent disability. Benign MS can only be identified when there is minimal disability 10 to 15 years after onset and initially would have been categorized as relapsing-remitting MS. Benign MS tends to be associated with less severe symptoms at onset (e.g. sensory); frequency: 15% to 25% (Compston and Coles, 2002, www.msif.org, 2004).
5. Progressive-relapsing: Gradual neurological deterioration from the onset of symptoms but with subsequent superimposed relapses; frequency: up to 15% (Compston and Coles, 2002).

#### 1.2.1.3 Etiology of multiple sclerosis

Although MS is one of the better studied AIDs, the etiology is still unresolved. The geographic heterogeneity of the disease and the widely varying prevalence rates in different ethnic populations suggest interplay between environmental and genetic factors. Alteration in the risk of acquiring MS among children of immigrants has been shown in migration studies (Kurtzke and Hyllested, 1987, Ghadirian et al., 1998). People who move from an area where the disease is common to an area where it is rare show a decreased risk, while migrants who move in the opposite direction tend to acquire a higher risk for developing MS. The decisive age for migration seems to be late puberty.

##### 1.2.1.3.1 Genetic susceptibility to multiple sclerosis

The idea that genetic factors may have a role in MS was first raised in the 1890s with the identification of familial clustering (Dyment et al., 2004). Studies document that family members of affected patients have an increased risk of developing MS, with first-degree relatives and daughters of affected mothers having the highest risks. Twin studies provide further evidence supporting the role of genetic background in MS (Perron and Seigneurin, 1999; fig. 3). Several potential predisposing loci for MS have been described in the meantime (Saarela et al., 2002, Haines et al., 2002, Kantarci et al., 2003).

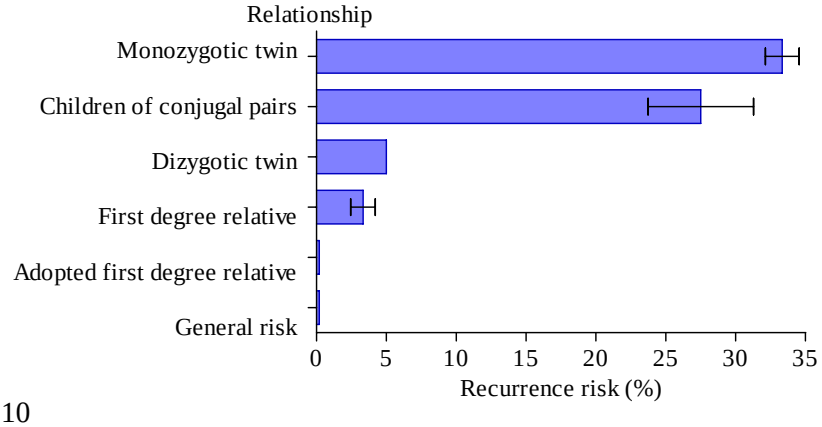


**Fig. 3: Selected familial recurrence risk for MS.** Lifetime prevalence in the general population is estimated 0.2%. (Dyment et al., 2004, Perron and Seigneurin, 1999, Compston and Coles, 2002).

The difference in concordance rate between dizygotic (DZ) and monozygotic (MZ) twins clearly argues for genetic influences. However, the concordance in MZ twins is only partial, implying that not a single gene but rather several genes contribute to MS susceptibility, together with non-genetic factors (Perron and Seigneurin, 1999). Age at onset, gender, and parental MS status seem to influence sibling risk (Montomoli et al., 2002). A maternal effect was indicated by half-sibling-studies, the cause for this maternal effect is not known, but could be genetic (e.g. mitochondrial genes), epigenetic (e.g., imprinted genes), or environmental (intrauterine or perinatal; Giordano and Momigliano-Richiardi, 2004). The risk is not increased either for individuals adopted into a family with an affected individual or in the non-biological relatives of adoptees who themselves develop MS (Compston and Coles, 2002).

#### 1.2.1.3.2 Ethnicity and multiple sclerosis

The first epidemiological study of MS already pointed out that the disease affected persons of Scandinavian decent more than other ethnic groups (Davenport, 1922). The 'Viking gene'-hypothesis – first published by Charles Poser and Sten Fredrickson (McDonald, 2002) – could at least in part explain the higher frequency of MS in people of Northern European descent. In fact, MS has never been reported in ethnically pure Inuits, North and South Amerindians, Australian Aborigines, New Zealand Maoris, Pacific Islanders or Lapps (Poser, 1994). However, there are several exceptions to this rule. The MS prevalence in several Canadian Indian tribes (Algonkian tribes, Cree, and Ojibway) is higher than the general prevalence in Canada (134 per 100,000 compared to 80 per 100,000 inhabitants; Mirsattari et al., 2001). Although prevalence rates in Africa are generally low, epidemics of MS have been reported in the 1980s (Rosman et al., 1985, Adam, 1989). Another exception is the high prevalence on the island of Sardinia, which was never invaded by the Vikings and whose population is genetically quite distinct from the Italian or European population (Cocco et al., 2004).

#### 1.2.1.3.3 Environmental risk factors in multiple sclerosis

Many risk factors for developing MS have been identified; among these are dietary habits which characterize over-nutrition (Ghardirian et al., 1998), smoking (Franklin and Nelson, 2003), low soil selenium, and iodine concentration, exposure to some heavy metals (Ghardirian et al., 1998), organic solvents (Riise et al., 2002), as well as other AIDs and migraine (Zorzon et al., 2003). Geographical clusters of MS were attributed to high latitude, endemic goiter, seasonality (Ghardirian et al., 1998, Willer et al., 2005) and climatic factors (Koziol and Feng, 2004). Increased risk was also described among persons who spent their childhood in an environment with a high level of hygiene (Lutton et al., 2004) and who have higher socioeconomic status (Ghardirian et al., 1998). Although all of these factors could contribute to developing MS, none has been clearly identified as causative agent.

#### 1.2.1.3.4 Infectious agents and multiple sclerosis

Epidemiological studies are still the strongest indication for an infectious etiology of MS. As early as 1894 Pierre Marie, a former student of Charcot, argued strongly that infection was the cause for MS (cited in Murray, 2002). Since then, many studies have suggested an association between MS development and/or episodes of exacerbation and concomitant infections. The possible virus-associated etiology for MS is based on (1) epidemiological evidence of childhood exposure to infectious agents and increase in disease exacerbations with viral infection; (2) geographic association of disease susceptibility with evidence of MS clustering; (3) evidence that migration to and from high-risk areas influences the likelihood of developing MS; (4) abnormal immune response to a variety of viruses, and (5) analogy with animal models and other human diseases in which viruses can cause diseases with long incubation periods, relapse, and demyelination (Lutton et al., 2004). The 'polio hypothesis' describes an increased risk in individuals exposed for the first time to a childhood infection after adolescence (Hernan et al., 2001) as suggested for EBV infections in MS (Haahr et al., 1994, Yamano et al., 2004). The agent(s) causing MS may trigger an autoimmune process without necessarily invading the nervous system ('hit and run' hypothesis; Hellings et al., 2004). The 'dual infection hypothesis' suggests that infection with one retrovirus is insufficient for the development of MS, and dual infections, such as a retrovirus and EBV, are required (Munch et al., 1997, Haahr et al., 1994). MS attacks have been associated with upper respiratory infections (URIs; Kriesel et al., 2004) and gastrointestinal infections (Compston and Coles, 2002). More recently, attention has focused on HHV-6, EBV, and the potentially MS-associated retroviruses (Tuke et al., 2004). Especially for pediatric MS an age-dependent relationship between EBV and MS development was suggested (Levin et al., 2005, Alotaibi et al., 2004). Antibodies to HTLV proteins (Shirazian et al., 1993), as well as expression of the tax sequence (a regulatory HTLV protein, Ferrante et al., 1997) have been described in samples from HTLV-negative MS patients. The presence of cytopathic effect and reverse transcriptase activity in cultures of peripheral blood mononuclear cells was described in 2 of 15 patients with MS and none of healthy controls (Kam-Hansen et al., 1989). Molecular mimicry between the $MBP_{96-106}$ epitope, a candidate autoantigen for MS, and the U24 protein of HHV-6 (residues 4 to 10) has been shown (Tejada-Simon et al., 2003). A recent study demonstrated the induction of CNS autoimmune disease by viral delivery of an epitope from *Haemophilus influenzae* (Croxford et al., 2005).

#### 1.2.1.3.5 Vaccination and multiple sclerosis

Besides viral infection, different vaccinations have also been implicated in the etiology of MS: rabies (Poser, 1986, Piyasirisilp et al., 1999), measles (Zorzon et al., 2003) as well as measles, mumps and rubella, influenza, and HBV (Atkins et al., 2000, De Keyser et al., 1998).

However, MS may be divided into at least five disease subtypes (pathology of MS; 1.2.1.2) and it is possible that none or only some of these have viral involvement.

#### 1.2.1.4 Diagnosis/prognosis of multiple sclerosis

Diagnostic criteria for MS comprise the following: (1) neurological symptoms typical for MS, (2) oligoclonal IgG bands indicating a chronic humoral immune response, (3) six lesions typical of MS on MRI, (4) one gadolinium-enhancing MRI lesion, and (5) the exclusion of other diseases that might cause the given symptoms in individuals younger than 50 years old (Petereit and Heiss, 2002).

It is necessary to provide clinical diagnostics in addition to MRI and CSF studies, since these are non-specific and cannot be used for differential diagnosis of MS and disseminated encephalomyelopathy, Devic's syndrome, HAM/TSP, nervous system AIDS, neurobrucellosis, Lyme disease, neusosarcoidosis, chronic myalgic encephalomyelitis, nervous system lupus or arteritis (Poser, 1994).

The Expanded Disability Status Scale (EDSS) achieved widespread use as a single measure of the severity of MS. Spontaneous recovery is rare when neurological deficits have persisted for longer than six months, and there are no known therapies that promote regeneration and reverse fixed neurological deficits (Rudick et al., 1997). The median disease course is 30 years (Pender and Wolfe, 2002). Studies have not definitively concluded whether or not there is an association between numbers of clinical relapses and long term outcome (Dyment et al., 2004). Several clinical markers predict a more severe progression of MS: progressive disease from the onset of symptoms, motor and cerebellar signs at presentation to neurologist, short interval between two relapses, poor recovery from relapse, and multiple cranial lesions at presentation (Rudick et al., 1997). There is a reduction in relapse rate for each trimester of pregnancy, but with about a 3-fold higher risk in the puerperium and no net effect on pregnancy on relapse rate (Compston and Coles, 2002).

#### 1.2.1.5 Treatment of multiple sclerosis

There is no drug that can cure MS; the aim of treatment is to reduce the frequency of relapses, limit the lasting effect of relapses, relieve symptoms, prevent disability arising from disease progression, and promote tissue repair (Compston and Coles, 2002). There are currently no effective treatments for primary progressive MS. Many therapies that are effective in the animal model, experimental autoimmune encephalomyelitis (EAE), are either ineffective in MS or actually make MS worse. Another consideration concerns the necessary extended safety throughout prolonged administration which is required (Pender and Wolfe, 2002). Different categories of drugs are used for MS treatment; these include antivirals such as interferon beta (e.g. Rebif), immunosuppressive/immunomodulatory agents such as Mitoxantrone, Azathioprine, Cyclophosphamide, Metotrexate, Cyclosporin, statins and intravenous immunoglobulin, corticosteroids and others such as Glatiramer

acetate. A variety of immunomodulatory and immunosuppressive agents are used to attempt a decrease of disease activity in those with active disease despite standard immunomodulatory therapy (Jeffery, 2004). Antibiotics, various monoclonal antibodies, and estriol are also being studied as possible therapeutic agents (Rizvi and Bashir, 2004) Remyelination strategies are largely at a preclinical state. Also deployed is DNA vaccination with naked DNA encoding chemokines and TNF alpha (Hellings et al., 2004). Hematopoietic stem cell transplantation is also performed, using either allogeneic or autologous transplantation, wherein the autologous transplantation carries a lower mortality but also a higher likelihood of persistent contamination of the patient's immune system with autoaggressive cells (Mancardi et al., 2005, Hellings et al., 2004, Zhang et al., 2002)

#### 1.2.1.6 Animal models for multiple sclerosis

Virus and animal models include experimental autoimmune encephalomyelitis (EAE, Lutton et al., 2004) which may be induced in a number of animal species. Other virus models are Theiler's murine encephalomyelitis virus (TMEV) infection of mice (Olson et al., 2001, Oleszak et al., 2004) JC virus (JCV, Stoner, 1993), and an alternative model of virus-induced demyelination, Semliki Forest virus (SFV) infection of mice (Atkins et al., 2000).

### 1.2.2 Scleroderma

Scleroderma, or systemic sclerosis (SSc), is a multi-organ chronic connective tissue disorder generally classified as one of the autoimmune rheumatic diseases. The word 'scleroderma' comes from two Greek words: 'sclero' meaning hard, and 'derma' meaning skin. Hardening of the skin is one of the most visible manifestations of the disease. Systemic sclerosis is a relatively rare disease (incidence 1 to 2 per 100,000 person-years), but is associated with significant mortality, the median survival time being approximately 11 years (Lambe et al., 2004, Mayes et al., 2003, Valentini and Black, 2002).

#### 1.2.2.1 Epidemiology of scleroderma

The highest incidence of systemic sclerosis occurs between 30 and 55 years of age (Artlett et al., 1998, Chen et al., 2003, Herrick and Worthington, 2002); the peak age of onset ranges from 45 to 54 years in white women and from 34 to 44 years in black women (Mayes, 2003, Valentini and Black, 2002). Scleroderma disproportionately affects women; female : male ratios range from 3:1 up to 14:1 (Artlett et al., 1998, Allcock et al., 2004, Lambe et al., 2004, Chen et al., 2003, Herrick and Worthington, 2002). Sex- and race-specific prevalence estimates were significantly higher for women than for men and for Blacks than for Whites in the US. Population studies indicate that SSc occurs more frequently in the US than in continental Europe, the UK, and some areas in Asia (Mayes et al., 2003, Mayes 2003; tab. 1).

**Tab. 1: Gender distribution, incidence, and prevalence rates of scleroderma**

| Geographic region | Female/male ratio | Annual incidence (per million inhabitants) | Prevalence (per million inhabitants) | Reference |
|---|---|---|---|---|
| Australia | | | 208 | (a) |
| France | | | 158 | (b) |
| France (Europeans) | | | 140 | (b) |
| France (Non-Europeans*) | | | 211 | (b) |
| Iceland | | 3.8 | 71 | (a) |
| South Australia | 4 : 1 | 16 | 233 | (c) |
| Japan, Tokyo | 14 : 1 | | 38 | (c) |
| UK | | 3.7 – 4.0 | 31 – 88 | (a), (d), (c) |
| USA | 3 : 1 | 4.5 – 20 | 28 – 286 | (a), (e), (f), (g), (c) |

* Non-Europeans: northern and sub-Saharan African, Asian, and Caribbean ancestries; References: (a) (Valentini and Black, 2002); (b)(Le et al., 2004); (c)(Chen et al., 2003); (d)(Allcock et al., 2004); (e)(Mayes et al., 2003); (f)(Mayes, 2003); (g)(Herrick and Worthington, 2002).

#### 1.2.2.2 Pathology of scleroderma

The pathogenesis of SSc is characterized by alterations in the microvasculature, excessive fibrosis, microvascular abnormalities and alterations in the immune system (Sawaya et al., 2004, Kuwana et al., 2004). The vasculopathy in the disorder mainly affects small arteries and capillaries and causes reduced blood flow and tissue ischemia, which lead to clinical manifestations such as Raynaud's syndrome, fingertip ulcers, and gangrene (Artlett et al., 1998). Progressive vascular defects, gastrointestinal involvement, and renal and pulmonary diseases account for early mortality (Kahaleh, 2004, Mayes, 2003).

#### 1.2.2.3 Etiology of scleroderma

The variations shown between different ethnic groups and genders support the hypothesis that the observed different susceptibilities for systemic sclerosis are due to genetic and/or environmental factors.

##### 1.2.2.3.1 Genetic susceptibility to scleroderma

Family studies indicate an increased risk for relatives to develop scleroderma. The relative risk for first-degree relatives to develop SSc was described to be 1.4% to 1.6% compared with 0.026% risk for the general population (Herrick and Worthington, 2002). Twin studies indicate that genetic factors are not sufficient to explain the development of SSc. Concordance for SSc was found to be similar in MZ and DZ twins with a relatively low overall concordance (4.5%; Feghali-Bostwick et al., 2003). Thus, in addition to genetic components, environmental co-factors must be involved in triggering scleroderma.

#### 1.2.2.3.2 Ethnicity and scleroderma

The prevalence of SSc is not equally distributed among ethnicities. Blacks are affected twice as frequently as Whites. Limited data for Japanese, Hispanic, and Native American populations suggest that they have more severe disease than Whites. The highest rate was seen in a genetically isolated population of Choctaw Native Americans, with a prevalence of 469 per 100,000 inhabitants (Reveille, 2003, Valentini and Black, 2002, Herrick and Worthington, 2002). The higher prevalence observed for non-Europeans supports the hypothesis of an ethnic factor involved in the pathogenesis of systemic sclerosis due to genetic factors.

#### 1.2.2.3.3 Environmental risk factors in scleroderma

Several environmental risk factors have been described for developing scleroderma. Exposure to specific organic solvents has been described as a risk factor for women (Garabant et al., 2003) and men (Bovenzi et al., 2004) in conflicting studies. Miners (coal, gold, silica, uranium) are at increased risk of developing scleroderma-like illnesses. Other possible risk factors include silica and certain drugs (appetite suppressants, bleomycin, carbidopa, cocaine and pentazocine). It has been proposed that hormonal factors or exposure to persisting fetal cells in maternal blood following pregnancy – microchimerism – may be involved in the pathogenesis (Sawaya et al., 2004, Artlett et al., 1998). Microchimerism has been detected in many organs, including the skin, in women with SSc (Johnson et al., 2001) but could not be confirmed by others (Selva-O'Callaghan et al., 2003, Pisa et al., 2002).

#### 1.2.2.3.4 Infectious agents and scleroderma

There is some evidence to suggest that SSc might be induced by either asymptomatic or common viral infections. For example, Parvovirus B19 infection was detected in the bone marrow of 12 out of 21 SSc patients (Ferri et al., 1999). Antibodies to retroviral Gag protein were detected in 33% of patients with mixed connective tissue disease, while no unaffected control subject had detectable levels of these antibodies (Hishikawa et al., 1997). So far no infectious causing agent can be definitely linked to SSc, but endogenous retrovirus sequences have been suggested to be important in the etiology and pathology of SSc (Talal et al., 1992).

### 1.2.2.4 Diagnosis/prognosis of scleroderma

A diagnosis of scleroderma is based largely on the medical history and the physical exam. Factors negatively affecting survival include male sex and older age at diagnosis (Mayes et al., 2003). Diffuse disease also seems to occur more commonly in the black population than in the white. Age at onset of diffuse disease is younger, on average, than the onset of limited disease (Mayes et al., 2003). A number of factors have long been known to be associated with a poorer survival, including internal organ involvement clinically manifest at presentation, diffuse disease, black race, male sex and older age at onset. At present, the

probability of survival for the scleroderma patient ranges from 70% to 95% at 4 to 7 years (Valentini and Black, 2002). SSc with involvement of vital organs has up to 50% 5-year mortality (Tyndall and Matucci-Cerinic, 2003).

#### 1.2.2.5 Treatment of scleroderma

Since the cause remains yet unknown, therapies are directed at improving peripheral blood circulation with vasodilators and antiplatelet aggregation drugs, at preventing the synthesis and release of harmful cytokines with immonsuppressant drugs, and at inhibiting or reducing fibrosis with agents that reduce collagen synthesis or enhance collagenase production (Sapadin and Fleischmajer, 2002).
More than 100 SSc patients have had autologous stem cell transplantation (ASCT); the majority of patients showed improvement of symptoms, the transplantation-related mortality was 9% to 17% (Tyndall and Matucci-Cerinic, 2003, Gratwohl et al., 2001).

### 1.2.3 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is considered to be the prototypic systemic autoimmune disease. It may affect any organ of the body. SLE displays a broad spectrum of clinical and immunological manifestations and may encompass a multitude of phenotypes.

#### 1.2.3.1 Epidemiology of SLE

The first known probable case of SLE was diagnosed in a mummy from the Huary culture in Peru from about 890 AD (Gamarra et al., 2004). A compilation of gender distribution, incidence, and prevalence for various regions and countries reveals biased distribution (tab. 2); nevertheless, no clusters or ‘hot spots’ of SLE could be determined (Petri, 2002). The overall prevalence of SLE is roughly ~1:2000 and is about the same as for MS and about 5- to 10-fold lower than type 1 diabetes mellitus or rheumatoid arthritis (Kotzin, 1996).

**Tab. 2: Compilation of gender distribution, incidence, and prevalence of SLE**

| Region | Female/male ratio | Annual incidence per 100,000 inhabitants | Prevalence per 100,000 inhabitants | References |
|---|---|---|---|---|
| Australia, Queensland, indigenous people* | | 92.8 | | (a) |
| Australia, Queensland, overall population ** | | 45.3 | | (a) |
| Brazil | | 8.7 | 8.7 | (b) |
| Curacao | 7.2 | 4.6 | 47.6 | (b), (c) |
| Denmark | | 3.6 | 21.7 | (b) |
| Greece | 7.4 | 1.9 | | (d) |
| Iceland | 6.3 | 3.3 | | (b) |

| Region | Female/male ratio | Annual incidence per 100,000 inhabitants | Prevalence per 100,000 inhabitants | References |
|---|---|---|---|---|
| India & Pakistan | | 18.9 | | (c) |
| Norway | 7.7 | 2.6 | 44.9 | (b) |
| Puerto Rico | 19.0 | | | (e) |
| Spain, Asturia | Up to 50:1 | 2.15 | 34.12 | (f) |
| Sweden | 5.5 | 4.0 – 4.8 | 68 | (b) |
| UK | 4.4 – 13.6 | 3.8 – 4.0 | 24.7 | (b) |
| USA | 8.8 – 9.8 | 1.0 – 7.6 | 5.8 – 130 | (b), (g) |
| Caucasians | 9.8 | | | (b), (g) |
| African-Americans | 4.6 | | | (b), (g) |
| USA, Gainesville GA | | 63.7 | 1000 | (b) |
| USA, Native American Indians*** | | 16.6 – 27.1 | | (c) |
| USA, Pennsylvania, African-American | 15.0 | 5.3 | | (c) |
| USA, Pennsylvania, overall | 11.6 | 2.4 | | (c) |
| USA, Pennsylvania, Whites | 10.8 | 2.0 | | (c) |

References: (a)(Bossingham, 2003); (b)(Petri, 2002); (c)(McCarty et al., 1995); (d)(Alamanos et al., 2003); (e)(Mayor and Vila, 2003); (f)(Lopez et al., 2003); (g)(Rider and Abdou, 2001).

* Indigenous Australian population

** Including roughly 10% Australian Aboriginal or Torres Strait Islander decent

*** Reported among 3 Native Indian tribes with shared ancestry and geography

Several studies indicate an increasing prevalence, probably due prolonged survival of SLE patients since the 1950s and 1960s (Alamanos et al., 2003). Data regarding the SLE prevalence in the USA compared to Europe, however, are conflicting. While some studies describe a higher prevalence in the USA compared to Scandinavia or the UK (Petri, 2002), others describe SLE to be more common in Europe than in the USA (McCarty et al., 1995).

#### 1.2.3.1.1 Gender related characteristics of SLE

The female to male ratio is greatest (>8:1) for patients presenting between ages 15 to 50 years, whereas the ratio is closer to 2:1 for disease that develops during childhood or after menopause (Kotzin, 1996, Lahita, 1999). Male lupus differs from female lupus in several laboratory characteristics and clinical presentations. Male patients have generally a poorer disease outcome, presenting higher disease damage and mortality (Petri, 2002, Mayor and Vila, 2003).

#### 1.2.3.1.2 Onset of SLE

Although SLE can occur nearly at any age, women of childbearing age are primarily affected (Kotzin, 1996). Age at onset or diagnosis varies quite dramatically among races and between countries. While there is a strong suggestion in Scandinavian studies of a later age at onset, the median age at SLE onset was significantly lower in Caucasian females

than in males (5 to 10 years) and in African-Americans compared to Caucasians (Cooper and Stroehla, 2003, Lopez et al., 2003, Petri, 2002). The mean age at diagnosis for white females is 39.8 years, compared to 35.2 years in African-American females (McCarty et al., 1995).

#### 1.2.3.2 Pathology of SLE

Systemic lupus erythematosus is a chronic, remitting, relapsing, inflammatory, and often febrile disorder of connective tissue, of acute or insidious onset; it is characterized by involvement of the skin, joints, kidneys, and serosal membranes. SLE can affect many parts of the body, including the joints, kidneys, heart, lungs, blood vessels, and brain. Common symptoms of SLE are painful or swollen joints (arthritis) and muscle pain, unexplained fever, kidney problems, red rashes, most commonly in the face (butterfly or malar rash), chest pain upon deep breathing, unusual loss of hair, pale or purple fingers or toes from cold or stress, sensitivity to the sun, swelling (edema) in legs and around eyes, mouth ulcers, swollen glands, extreme fatigue, and anemia. The SLE disease activity index (SELDAI) is used as a single measure of the severity. The risk of myocardial infection in young women with SLE is increased 52-fold over the general population (Petri, 2002). Preterm deliveries are associated with SLE disease activity (Clark et al., 2003). Cognitive dysfunction represents one of several neurological and psychiatric complications of SLE. Additional manifestations of nervous system involvement subsumed under the term ‘neuropsychiatric SLE’ (NPSLE) include cerebrovascular disorders, seizures, psychosis, acute confusional states, anxiety, and mood disorders (Sweet et al., 2004).

#### 1.2.3.3 Etiology of SLE

Studies of familial clustering, ethnic distribution, and influence of environmental factors, suggest that SLE is of multifactorial and multigenic origin.

##### 1.2.3.3.1 Genetic susceptibility to SLE

SLE clusters significantly within families of Caucasian SLE patients as well as in the families of Taiwanese patients (Corporaal et al., 2002, Huang et al., 2004). Lahita et al. observed father to son transmission and noted prepubertal onset of familial SLE in males (Lahita et al., 1983). Twin studies showed a higher concordance for clinical and serological abnormality for monozygotic twins, indicating a significant genetic factor (Block et al., 1975). The apoptosis genes FAS and FASL are candidate contributory genes in human SLE (McNally et al., 1997), although there are conflicting results (Kojima et al., 2000). A genome-wide search for susceptibility genes supports the hypothesis that multiple genes, including one in the HLA region, influence susceptibility to human SLE (Gaffney et al., 1998).

#### 1.2.3.3.2 Ethnicity and SLE

Different ethnic groups exhibit quite different SLE rates (tab. 2). SLE has long been known to be more prevalent among US Blacks, in whom the disease tends to have an earlier onset and a more severe form (Molina and Ehrenfeld, 2003). At least a 3- to 5-fold increase in SLE incidence among African-American females compared with white females, and a 2-fold increase in incidence among African-American males compared with white males has been observed (McNally et al., 1997). Age at diagnosis is approximately 7 years younger among Blacks compared with Whites. As African-Americans, African-Caribbeans have a highly increased incidence of SLE versus Caucasian Americans (Petri, 2002, McCarty et al., 1995). In contrast, connective tissue disorders such as SLE are uncommon in Africa. Studies from Kenya, Nigeria, South Africa, Uganda, and Zimbabwe have all independently noted that SLE is rare (Taylor and Stein, 1986, Adebajo, 1992). Studies conducted in Hawaii, New Zealand, Malaysia, and China showed frequency of SLE among Asian and Pacific Islanders to be higher than that in Whites (McCarty et al., 1995). SLE is common in China (Chang, 1983), Malaysia (Frank, 1980), India (Malaviya, 1986), Puerto Rico (Mendez-Bryan et al., 1964) and the West Indies (Harris et al., 1989). In Malaysia, those of Chinese ethnic origin seem to be more vulnerable to SLE than Malays or those of Indian origin (Frank, 1980, Wang et al., 1997). In contrast, no difference was found between Whites and Asians in San Francisco (McCarty et al., 1995). Lupus is more frequent in Aborigines than in Caucasians in Australia (Petri, 2002). The incidence rates reported in southern Sweden, Iceland, and the UK have been similar. The highest rates of SLE have been reported among 3 Native American tribes with shared ancestry and geography (17 to 27 per 100,000; McCarty et al., 1995). An increased risk of SLE has also been reported among Asian and Afro-Caribbean immigrants in the United Kingdom (Cooper and Stroehla, 2003, Corporaal et al., 2002).

#### 1.2.3.3.3 Environmental risk factors in SLE

The increased frequency of SLE in women indicates the possible involvement of hormones in the etiology. Some studies have found that exogenous exposure to estrogen, either through oral contraceptives or estrogen replacement therapy, may increase the incidence of SLE, and changes of disease activity have been observed after administration of exogenous hormones (Rider and Abdou, 2001, Petri, 2002). Cigarette smoking is associated with increased disease activity in SLE (Ghaussy et al., 2003), as well as with higher disease frequency (Formica et al., 2003). Certain drugs, such as alpha-methyldopamine, hydralazine, procainamide, and antiepileptics, are able to elicit so-called drug-induced SLE. It is also clear that chronic treatment of patients with certain drugs can induce the production of anti-nuclear antibodies and a lupus-like disease (Kotzin, 1996). Heavy metals such as mercury are also able to induce an SLE-like syndrome and nephritis in rats. Sunlight not only acts as a trigger for lupus but can also worsen the course of the disease. Findings suggest that dietary nutrients may modify the clinical course of disease in female

patients with SLE; for example, Vitamin C intake is inversely associated with the risk of active disease (Minami et al., 2003). Exacerbation of the disease is often seen after bacterial or viral infections.

#### 1.2.3.3.4 Infectious agents and SLE

As for most, if not all, slowly progressing diseases, different infectious agents have been suspected to play a role in SLE disease development. In the 1880s tuberculosis was suspected to be a causative agent (Gamarra et al., 2004). A history of shingles and of hives was found to be associated with SLE (Strom et al., 1994). More recently, association of HERVs regarding the etiology and pathology of SLE has been discussed (Herrmann et al., 1996, Ranki et al., 1992); especially HERV-E was suggested to play a role (Hishikawa et al., 1997). Detection of antibodies reactive with different segments of ERV-9, HERV-H (Bengtsson et al., 1996), ERV-3 (Li et al., 1996), intracysternal type A particles (IAP), HTLV and with HIV (Nelson et al., 1994), have suggested the possibility that these represent cross-reactive antibodies to another retrovirus.

### 1.2.3.4 Diagnosis/prognosis of SLE

SLE is a chronic disease with a broad spectrum of clinical and immunological manifestations; therefore this condition may be frequently misdiagnosed. Patients may be classified as having SLE if they have four or more of the following eleven disorders: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological disorder, haematological disorder, immunological disorder, antinuclear antibodies. Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double-stranded or single-stranded DNA, nucleoprotein, histones, and nucleolar RNA. Autoantibodies are typically present many years before the diagnosis of SLE; the appearance of autoantibodies tends to follow a predictable course, with a progressive accumulation of specific autoantibodies before the onset of SLE, while the individuals are still asymptomatic (Arbuckle et al., 2003). Since the 1950s, the estimated 5-year survival of SLE patients in developed countries rose from <50% to >95% and similar increases were seen in the 10-year survival rates. However, mortality rates of SLE patients remain approximately 3 times that of age- and gender-matched controls (Borchers et al., 2004).

### 1.2.3.5 Treatment of SLE

At present there is no cure for SLE. Treatment plans have to be tailored to the individual's needs and may change over time. Nonsteroidal anti-inflammatory drugs (NSAIDs) are often used. Antimalarials are generally used to treat fatigue, joint pain, skin rashes, and inflammation of the lungs. The mainstay of SLE treatment involves the use of corticosteroid hormones, such as prednisone, hydrocortisone, methylprednisolone, and dexamthasone. Immunosuppressives may be used for some patients whose kidneys or central nervous system are affected by SLE. In some patients, antirheumatic drugs may be

used to help control the disease. Autologous stem cell transplantation (ASCT) following immunoablative chemotherapy was performed in 53 SLE patients. The study showed the efficacy of the ASCT for remission induction of refractory SLE, although the mortality rate due to the procedure appeared high (13%). The return of disease activity in one-third of patients might be reduced by long-term immunosuppressive therapy post-ASCT (Jayne et al., 2004). Other studies note that roughly half of the patients (47%) need immunosuppressive therapy in addition to corticosteroids after HSCT (Gratwohl et al. 2001).

#### 1.2.3.6 Animal model for SLE

Sun et al. reported that treatment with an antagonistic monoclonal antibody to CD137 blocked lymphadenopathy and spontaneous AID in Fas-deficient mice (a model for SLE) ultimately leading to their prolonged survival (Sun et al. 2002).

### 1.2.4 Spondylitis ankylosans

Spondylitis ankylosans (SA), also ankylosing spondylitis (AS), is one of many forms of inflammatory arthritis, the most common of which is rheumatoid arthritis, and belongs to the subgroup of spondylarthropathies (SpA). The SpAs are defined as inflammatory arthropathies characterized by sacroiliac involvement and relationship to HLA-B27. SA is the prototype of the SpAs and one of the most common rheumatic diseases. Paleopatholgocial studies of Egyptian mummies revealed that SA has afflicted humans since antiquity (Sieper et al., 2002). It is estimated that patients with SA have about a 50% increased risk of mortality (Braun and Pincus, 2002).

#### 1.2.4.1 Epidemiology of spondylitis ankylosans

The prevalence of SA worldwide is estimated to be 0.9% to 1.0% (Sieper and Braun, 2002, Braun et al., 1998); the prevalence of SA in European Caucasoid populations ranges from 0.2% to 0.9% (Zhang et al., 2004). Approximately 800,000 people in Germany, 3 million in the European community and 2.4 million in the US are affected (Braun et al., 1998). The prevalence of SA in Berlin was reported to be 0.86%; the reported adult SA prevalence in Finland was 0.15% and 1.1% to 1.4% in Norway (men 1.9% to 2.2%, women 0.3% to 0.6%; Sieper et al., 2002).

##### 1.2.4.1.1 Gender related characteristics of spondylitis ankylosans

Men are afflicted with SA approximately 2 to 3 times more frequently than women. Estimated percentages of male patients among the SA patient population range from 65% to 80% and vary by geographic location (68.9% in a German rheumatological database, n=8776, and 87.3% in a French study, n=473; Sieper et al., 2002, Zhang et al., 2004, Sieper and Braun, 2002). The disease tends to be more severe in men, which is also reflected by varying disease patterns (Jimenez-Balderas and Mintz, 1993). The spine and pelvis are most commonly affected in men, with some involvement of the chest wall, hips,

shoulders, and feet. In contrast, women have less severe involvement of the spine, with more symptoms in the knees, wrists, ankles, hips, and pelvis (Sieper et al., 2002). While most patients with rheumatoid arthritis experience sustained or increased improvement of the disease during pregnancy, patients with SA show a frequent increase in disease activity in the second trimester and mitigation of symptoms in the third trimester (Ostensen et al., 2004).

#### 1.2.4.1.2 Onset of spondylitis ankylosans

The distribution pattern of age at the time of first spondylitic symptoms in German SA patients shows that 4% are younger than 15 years old; 90% are between 15 and 40, and the remaining 6% are more than 40 years old. The mean age of onset in German SA patients is 28.3 years old. Juvenile onset (below 16 years of age) is known to occur particularly if environmental triggers are present. The clinical picture of juvenile onset differs from that of adult onset by more frequent involvement of peripheral joints, e.g. a striking increase in the prevalence of total hip replacements in those with juvenile onset AS (18%) compared to adult onset (8%). Thus patients within the most productive periods of life are affected (Sieper and Braun, 2002, Sieper et al., 2002).

### 1.2.4.2 Pathology of spondylitis ankylosans

The condition primarily causes inflammation of the joints between the vertebrae of the spine and the joints between the spine and pelvis (sacroiliac joints). It may also affect the joints in the ribs where they attach to the spine and the joints in arms and legs. The attachments of ligaments and tendons to joints (the entheses) are also frequently involved, as well as the eyes, and, less commonly, the heart and lungs. As the condition worsens and the inflammation persists, new bone forms in the healing process. The vertebrae begin to grow together, forming vertical bony outgrowths (syndesmophytes) and becoming stiff and inflexible. Fusion can stiffen the rib cage, restricting lung capacity and function. In advanced stages of the disease, the fusion typically ascends the spine, forming a long bony column referred to as ‘bamboo spine’. The most serious complication encountered in SA is spinal fracture; even minor trauma to the rigid, fragile spinal column can cause severe damage. Prostatis is highly prevalent among men with SA. Aortic insufficiency and cardiac conduction disturbances can occur in patients with long term disease. Amyloidosis, cauda equina syndrome, and pulmonary fibrosis are rare complications (Sieper et al., 2002).

### 1.2.4.3 Etiology of spondylitis ankylosans

Various risk factors have been described for spondylitis ankylosans, implying a multifactorial background for the etiopathogenesis.

#### 1.2.4.3.1 Genetic susceptibility to spondylitis ankylosans

Spondylitis is clearly linked to the class I MHC molecule HLA-B27 (principal function: T cell activation, Sieper and Braun, 2002): 90% of people affected by spondylitis ankylosans

have the HLA-B27 gene and about 2% of the people with this gene develop the condition. HLA-B27 varies for different ethnic groups and geographical regions; so far at least 23 subtypes have been identified. A significantly older average age at onset was observed for HLA-B27-negative individuals compared to HLA-B27 positives. The frequency of juvenile disease onset (before age 16) is independent of HLA-B27 status (Feldtkeller et al., 2003).

The non-concurrent development of SA in twins, especially in monozygotic twins (concordance rate of about 75%), suggests that environmental factors also may play a part in pathogenesis. Although no other genes have been proved to be responsible for SA, potential candidates include certain MHC class I and MHC class II genes, and non-MHC genes. Genome-wide screens have identified other susceptibility regions on several chromosomes (Sieper et al., 2002, Zhang et al., 2004).

#### 1.2.4.3.2 Ethnicity and spondylitis ankylosans

Prevalence appears to vary among ethnic groups; the global distribution shows high prevalence in certain Native American populations (Peschken and Esdaile, 1999, Walsh et al., 1998), the overall prevalence of SA among adult Inuit populations in two study regions in Alaska was estimated at 2.5% (Sieper et al., 2002). Otherwise, SA is basically a disease seen in Caucasians. Accordingly, high frequency of SA in the US is seen in Caucasians, followed by those with mixed Caucasian/Native Americans or Caucasian/eastern Asian heritage (Zhang et al., 2004). The estimated nationwide prevalence of SA among the total Japanese population (9.5/100,000) is less than 1/200 of that among white subjects (Sieper et al., 2002). Familial clusters of SA are reported in China (Liu et al., 2001).

#### 1.2.4.3.3 Infectious agents and spondylitis ankylosans

Bacterial infections are suggested to be triggering events in the pathogenesis of SA; frequent gastrointestinal (GI) infections constitute a risk factor for developing the disease (Sieper et al., 2002). The close relationship between SA and inflammation of the gut mucosa, associated with subclinical forms of inflammatory bowel disease (IBD), suggests that normal gut bacteria and, subsequently, immune reaction directed against gut bacteria, may also participate in the pathogenesis of SA (Duchmann et al., 1999). Enteric infections with *Klebsiella pneumoniae* and *Escherichia coli* have been implicated in the pathogenesis of SA in genetically susceptible hosts (Ebringer et al., 1978, Maki-Ikola et al., 1991, Sieper et al., 2002).

### 1.2.4.4 Diagnosis/prognosis of spondylitis ankylosans

In most cases, this complex disease is mild, and several years typically elapse before radiological techniques allow a definite diagnosis. Thus, diagnosis is often made only after significant structural damage has occurred (Sieper and Braun, 2002). Clinical assessment of disease activity is difficult in part due to the inaccessibility of the body areas usually involved, and overall because the correlation between the most frequently used serum

markers, non-specific biological indicators of inflammation, and the activity of the disease is controversial. High concentrations of inflammation mediators are present in only 34% to 64% of patients with severe disease (Munoz-Villanueva et al., 2003). On the basis of the 1984 modified New York criteria, the diagnosis of SA can be made by detection of sacroiliitis (by radiography, MRI, or computed tomography) in the presence of at least two clinical manifestations of spondylarthropathy (e.g. enthesitis and uveitis; Sieper et al., 2002).

#### 1.2.4.5 Treatment of spondylitis ankylosans

At present, treatment is aimed at relieving symptoms and improving function, but no currently approved treatment alters the natural course of the disease (Sieper and Braun, 2002). Physiotherapy plays an important role in preventing complications and physical deformities. Radiation therapy of the spine, performed quite extensively in previous decades, has been associated with a mean radiation dose of about double that of the atomic bomb survivors, an increased risk of leukemia and mortality, and has been largely abandoned today (Braun and Pincus, 2002). Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective as chronic SpA treatment, but have symptomatic rather than disease modifying effects (Sieper and Braun, 2002). Disease-modifying antirheumatic drugs (DMARDs) have been used to treat inflamed joints and other tissues, but have not been shown to be as effective in SA as they have been in RA (Sieper et al., 2002). Corticosteroids can be used to suppress inflammation and slow joint damage.

## 1.3 Retroviruses

Friedrich Löffler and Paul Frosch described the first virus (foot-and-mouth disease virus) in 1898. Walter Reed showed in 1901 that Yellow Fever was caused by a filterable virus, transmitted by mosquito bites (Reed et al., 2001). Shortly thereafter the first retrovirus was discovered in 1904 by Vallee and Carre, the equine infectious anemia virus (EIAV; Vallee and Carre, 1904). In 1908 Ellerman and Bang described a virus-induced leukemia in chickens (Ellermann and Bang, 1908) and Peyton Rous transmitted a tumor disease with a filterable agent, the Rous sarcoma virus, in 1910 (Rous, 1910). Thus, retroviruses were the first viruses shown to cause cancer. Although they were discovered at the beginning of the century, the characteristic feature of these viruses, the reverse transcription, was first described only in 1970 (Temin and Mizutani, 1970, Baltimore, 1970). A wide variety of retroviruses are known today that infect predominantly vertebrates, including primates and humans, but also fish, avian and insect retroviruses are known (Vogt., 1997, Arnaud et al., 2005). The clinical pictures vary widely, apart from unapparent infections, leukemias, lymphomas, sarcomas and other tumors (Essex et al., 1980, see addendum 5.6), immunodeficiencies, neuronal and autoimmune diseases have been observed (Matano, 1999). Retroviruses (reverse transcriptase oncoviruses) contain an RNA genome in the

virion but replicate through a DNA intermediate; the term ‘retro’ (backwards) is related to the fact that these viruses appear to transfer information backwards from RNA to DNA.

### 1.3.1 Retrovirus taxonomy

The taxonomy of the retroviridae is based on genetic, morphologic and antigenic properties, on host specificity, as well as characteristics of infection and pathogenesis. At present, seven genera have been defined: alpha-, beta-, gamma-, delta-, and epsilonretroviruses as well as lenti- and spumaviruses (van Regenmortel et al., 2000; fig. 4).

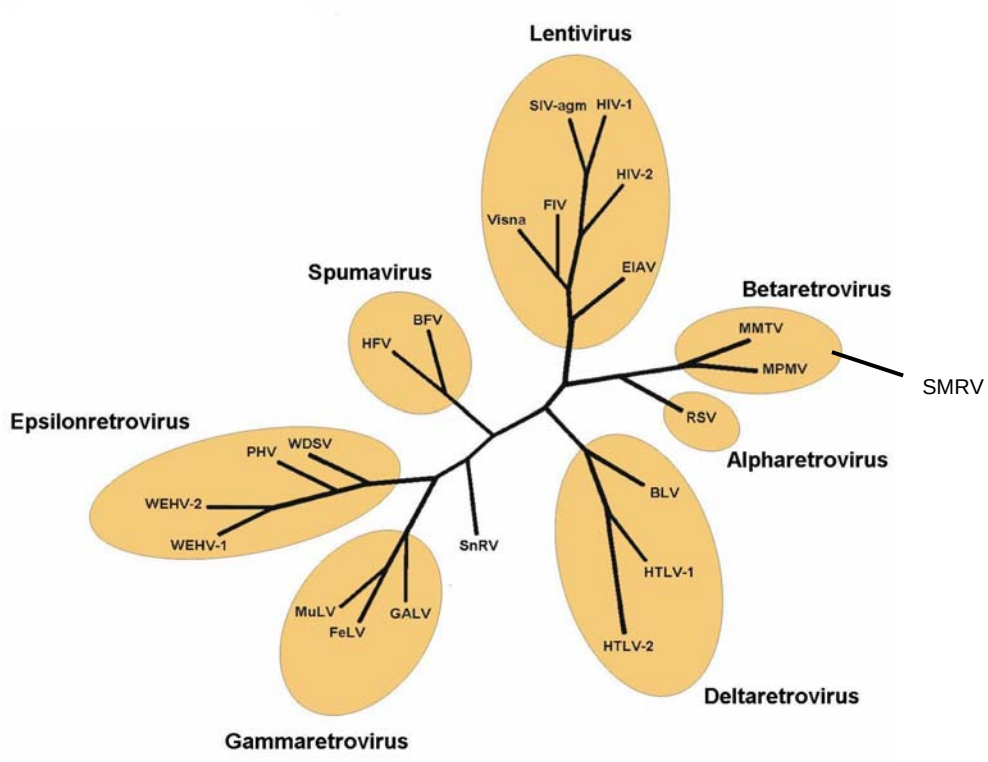


**Fig. 4: Taxonomic classification of exogenous retroviruses. Alpharetrovirus**: RSV: Rous sarcoma virus; **Betaretroviruses**: MMTV: Mouse mammary tumor virus, MPMV: Mason-Pfizer monkey virus; SMRV: Squirrel monkey retrovirus; **Gammaretroviruses**: MuLV: Murine leukemia virus, FeLV: Feline leukemia virus, GALV: Gibbon ape leukemia virus; **Deltaretroviruses**: HTLV-1/-2: Human T cell lymphotropic virus type 1/2, BLV: Bovine leukemia virus; **Epsilonretrovirus**: WEHV-1/-2: Walley epidermal hyperplasia virus, PHV: Perch hyperplasia virus WDSV: Walleye dermal sarcoma virus; **Lentiviruses**: Visna: Visna/Maedi virus, FIV: Feline immunodeficiency virus, HIV-1/-2: Human immunodeficiency virus type 1/2, SIVagm: Simian immunodeficiency virus, agm: African green monkey, EIAV: Equine infectious anemia virus, **Spumaviruses**: HFV: Human foamy virus, BFV: bovine foamy virus, **without classification**: SnRV: Snakehead retrovirus (modified according to van Regenmortel et al., 2000, Chiu and Skuntz, 1986).

### 1.3.2 Host range

The retrovirus surface (SU) envelope glycoprotein binds to a specific receptor on the surface of the host target cells to initiate infection. The specificity of this interaction does much to determine the cell tropism and pathogenesis of the respective virus, or even

isolates of the same virus (e.g. HIV). Retroviruses can be subdivided on the basis of receptor-determined host species specificity. Ecotropic viruses replicate in the cells of the host species and sometimes in those of closely related species. Xenotropic viruses are endogenous viruses, i.e. genetically acquired viruses that may be expressed from cells of a given animal but are unable to infect cells of that species. Xenotropic viruses may infect cells of many other species with varying efficiency. Amphotropic viruses are able to infect the cells of their host and the cells of other species.

### 1.3.3 Morphology of retroviruses

The virions are enveloped, slightly pleomorphic, and the spherical particles are 80 nm to 120 nm in diameter. The surface projections of the envelope are small (the surface appears rough) or distinct (8 nm long glycoprotein). The spikes are dispersed evenly on the surface (Vogt, 1997).

Retroviruses can be differentiated according to morphological characteristics using electron microscopy (Biel and Gelderblom, 1999, Gentile and Gelderblom, 2005; fig. 5). The images show members of different retrovirus families: betaretrovirus (MPMV), deltaretrovirus (HTLV), lentivirus (HIV), and spumavirus (FV).

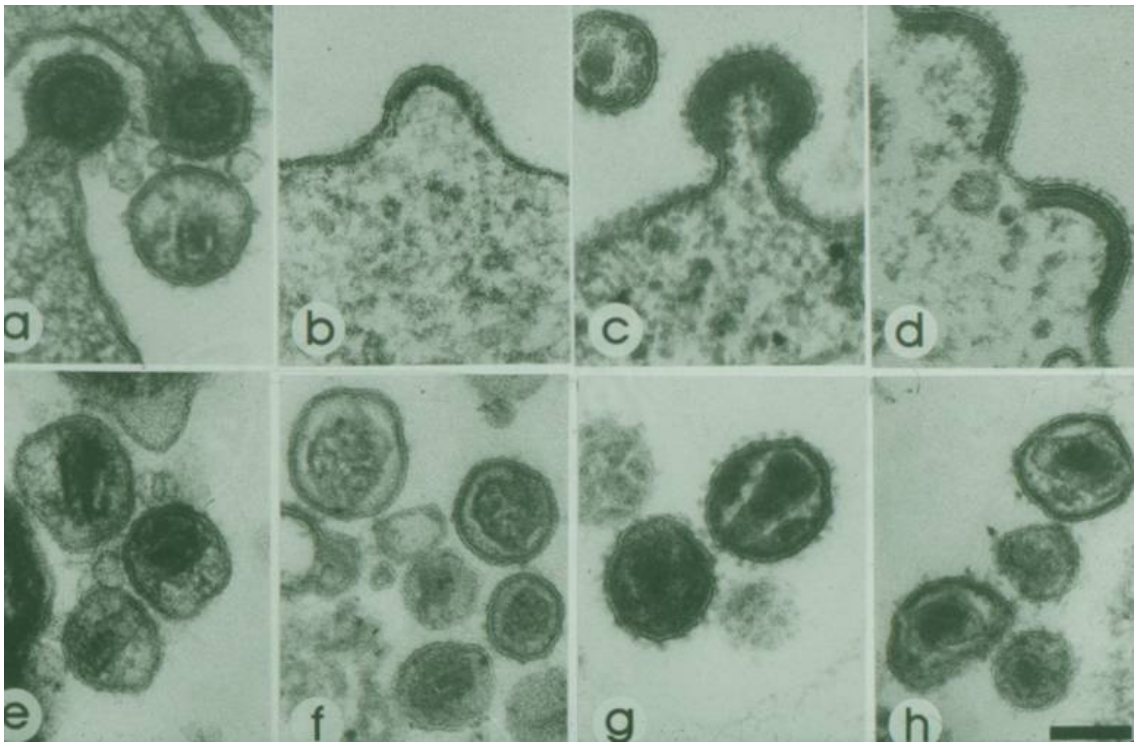


**Fig. 5: Electron microscopy: thin sectioning of retrovirus infected cells.** Above: budding of virus particles from the cell membrane, below: mature virus particles (uranyl acetate staining). a/e: Mason-Pfizer Monkey virus (MPMV), b/f: human T cell leukemia virus (HTLV), c/g: human immunodeficiency virus (HIV), d/h: foamy virus (FV; H. Gelderblom, RKI).

There are a number of virus proteins in the virus coat and typically seven internal proteins, four of which are structural and three enzymatic (fig. 6). These enzymatic activities found

in the virus particle are reverse transcriptase, DNA endonuclease (integrase), and protease. The virion also contains specific cellular tRNA molecules used as primer in replication.

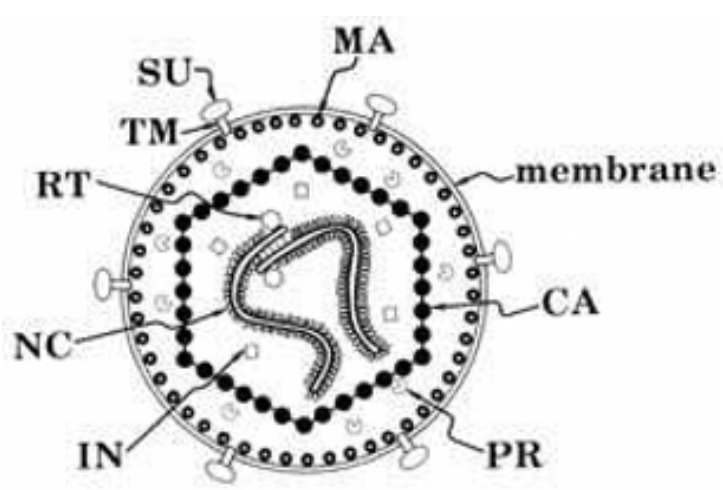


**Fig. 6: Scheme of a retrovirus particle.** The named proteins and their functions are listed in tab. 3. Only essential proteins are named. Some retroviruses also encode additional and non-essential proteins (simple/ complex retroviruses). The two RNA molecules are linked by hydrogen bonds (co-sediment). The capsid protein is the most abundant protein in the particle (~33% of the total weight). The outer envelope glycoprotein (SU) is linked by disulfide bonds to the transmembrane glycoprotein (TM).

Source: www. hmc.psu.edu

**Tab. 3: Retrovirus proteins and functions**

| | Protein | Function |
|---|---|---|
| MA | Matrix | Matrix protein (*gag* gene); lines envelope |
| CA | Capsid | Capsid protein (*gag* gene); protects the core; most abundant protein in virus particle |
| NC | Nucleocapsid | Capsid protein (*gag* gene); protects the genome; forms a core |
| PR | Protease | Essential for Gag protein cleavage during maturation (*pol* gene) |
| RT | Reverse transcriptase | Reverse transcribes the RNA genome (*pol* gene); also has RNaseH activity |
| IN | Integrase | needed for integration of the provirus (*pol* gene) |
| SU | Surface glycoprotein | Outer envelope glycoprotein (*env* gene); major virus antigen |
| TM | Transmembrane protein | Inner component of the mature envelope glycoprotein (*env* gene) |

According to Vogt, 1997

### 1.3.4 Retrovirus genome

Retroviruses contain a single stranded linear RNA genome in positive sense orientation. Two identical RNA genomes held together by hydrogen bonds are incorporated in a virus particle. The total genome of one monomer is 7000 to 12000 nucleotides in length. The 5'-terminus has a methylated nucleotide cap. The 3'-terminus of each monomer has a poly(A) tract and a tRNA-like structure. Simple retrovirus genomes harbor only three genes, *gag*, *pol*, and *env*, while complex retroviruses have accessory genes coding for regulatory proteins (e.g. *tax* in HTLV and *onc* in Rous sarcoma virus downstream from *env* which is involved in cellular transformation and cancer; fig. 7).

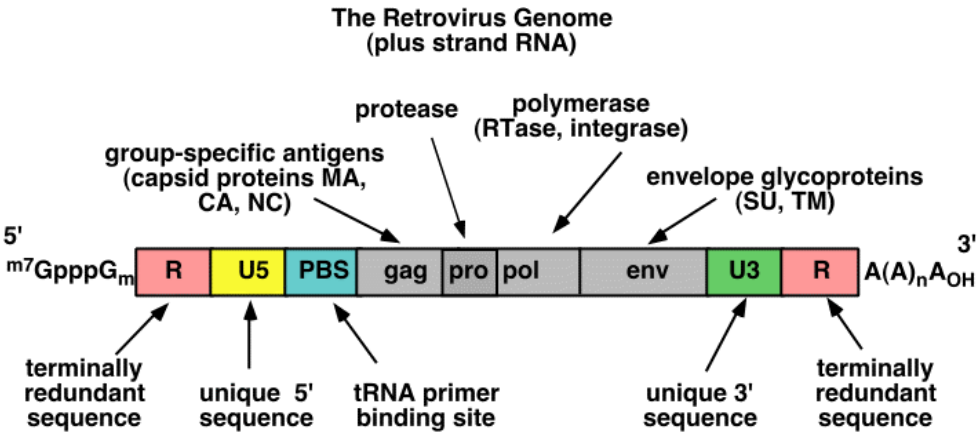


**Fig. 7: Scheme of a 'simple' retrovirus genome** (e.g. gammaretrovirus, MuLV, no regulatory genes in contrast to complex retroviruses). The lengths are not reflecting the actual proportions. The genome regions are specified in tab. 4. Source: www.bioinf.uni-leipzig.de.

**Tab 4: Retrovirus genome**

| Genome region | Characteristics |
|---|---|
| R-Region | A short (18 nt to 250 nt) sequence which forms a direct repeat at the both ends of the genome, which is therefore 'terminally redundant'. |
| U5 | A unique, non-coding region of 75 nt to 250 nt which is the first part of the genome to be reverse transcribed. |
| Primer binding site (PBS) | 18 nt complementary to the 3'-end of the specific tRNA primer used by the virus to begin reverse transcription. |
| Leader | A relatively long ( nt to 500 nt) non-translated region downstream of the transcription start site and therefore present at the 5'-end of all virus mRNAs. |
| Polypurine tract | A short (~10 nt) run of A/G residues responsible for initiating (+) strand synthesis during reverse transcription. |
| U3 | A unique non-coding region of 200 nt to 1,200 nt which forms the 5'-end of the provirus after reverse transcription; contains the promoter elements for transcription of the provirus. |

According to Vogt, 1997

Retrovirus genomes have several unique features: (1) retroviruses are the only viruses which contain two identical genomes in one particle (diploid viruses), (2) they are the only RNA viruses whose genome is produced by cellular transcriptional machinery (without any participation of a virus-encoded polymerase), (3) they are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication, and (4) they are the only (+) sense RNA viruses whose genome does not serve directly as mRNA immediately after infection.

### 1.3.5 Replication cycle

The replication cycle is shared among all infectious retroviruses. The HIV replication cycle is shown in fig. 8.

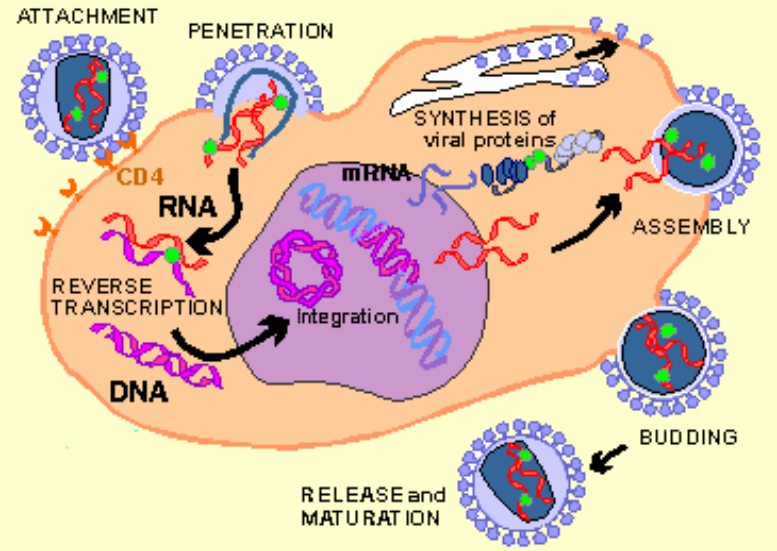


**Fig. 8: Replication cycle of HIV.** Source: http://web.uct.ac.za

The replication cycle starts by the binding of gp120 of HIV to CD4 on the target cell (attachment). The fusogenic domain in gp41 and fusin, a G-protein-linked receptor in the target cell membrane, mediate fusion. The nucleocapsid containing the viral genome and enzymes enters the cell (penetration), resulting in partial uncoating of the viral capsid. The viral genome and enzymes are released following the removal of core proteins. Then the viral reverse transcriptase catalyzes reverse transcription of ssRNA, forming RNA-DNA hybrids (reverse transcription). The original RNA template is partially degraded by ribonuclease H, followed by synthesis of a second DNA strand to yield proviral dsDNA. The generation of proviral cDNA results in two copies of the long terminal repeat (LTR) made up of the R, U3, and U5 regions. The viral dsDNA is then translocated to the nucleus, and integrated into the host chromosomal DNA by the viral enzyme integrase (integration). The matrix protein p17 enhances transportation of viral RNA into the nucleus, allowing HIV to infect resting cells (Valentin et al., 1990, von Schwedler et al., 1994). This capability distinguishes HIV from other retroviruses. Following infection, the provirus is activated by cellular transcription factors. The integrated provirus acts as its own gene that is transcribed from the viral promoter contained in the LTR. Transcription terminates at the other LTR at the end of the provirus. Genomic ssRNA, and, after processing several mRNAs, are exported into the cytoplasm. Host cell enzymes translate viral mRNAs into structural proteins and in the case of complex retrovirus, such as HIV, regulatory proteins as well. Immature capsids containing retrovirus ssRNA and proteins assemble beneath the host-cell membrane, into which transmembrane proteins TM (i.e. gp41) and surface proteins SU (i.e. gp120) are inserted (assembling). The membrane buds

out, forming the viral envelope (budding). The finals stages of capsid maturation occur in the virion by means of encapsidated protease after release from the infected cell.
The integration of the retroviral genome in the host genome by way of the DNA intermediate is being studied as a means of introducing foreign genes into a host, a prerequisite for gene therapy.

## 1.3.6 Exogenous and endogenous retroviruses

The ability of retroviruses to integrate into the host cell genome has provided a means for these viruses to colonize during evolution into the genome of virtually all vertebrates. Consequently, retroviruses are transmitted horizontally as ‘exogenous’ or vertically as ‘endogenous’ viruses (Spencer et al., 2003).

### 1.3.6.1 Endogenous retroviruses

#### 1.3.6.1.1 Origin of endogenous retroviruses

Some 45% of the human DNA is composed of transposable elements such as LINE or Alu retroelements and DNA transposons. Around 8% (~450,000 copies) of the genome is derived from sequences with similarity to retroviruses (Griffiths, 2001, Portis, 2002, Lander et al., 2001). Endogenous retroviruses could be derived from infectious retroviruses that entered the germ line, and were fixed in the population during evolution (Urnovitz and Murphy, 1996) but they could also be arisen from re-integrating retrosequences (Brosius, 1999). A summary of mobile elements in the human genome is added in the addendum (5.7). Screening of the human genome for human endogenous retrovirus (HERV) related sequences (not including flanking LTRs) resulted in ~7,800 HERV regions covering about 1.1% of the genome, with an average unit size of 4,300 nucleotides and ~38,000 retroviral open reading frames (ORFs; Villesen et al., 2004, Kim et al., 2004). Except for the evolutionary young HERV-K group (Tristem, 2000), most HERV families integrated into the primate genome approximately 30 to 45 million years ago (Sverdlov, 2000, Medstrand and Mager, 1998), and possibly up to a hundred million years ago (Shih et al., 1991). These data are based on genome comparisons of humans and primates. Measurement of the divergence of LTR sequences has been used as a ‘molecular clock’ to estimate the age of HERVs (given that the LTRs are identical at the time of integration). By analyzing HERV integration sites, the evolution of these elements has been tracked through the primate lineage: the split of old world monkeys (OWM) and new world monkeys (NWM) about 45 million years ago, OWM-ape split 30 million years ago, divergence of hominid from Old World monkeys >25 million years ago (Portis, 2002), and radiation of the great apes 15 million years ago (Shih et al., 1991, fig. 9). Thus, HERVs represent footprints of previous retroviral infection and have been termed ‘fossil viruses’ (Nelson et al., 2003).

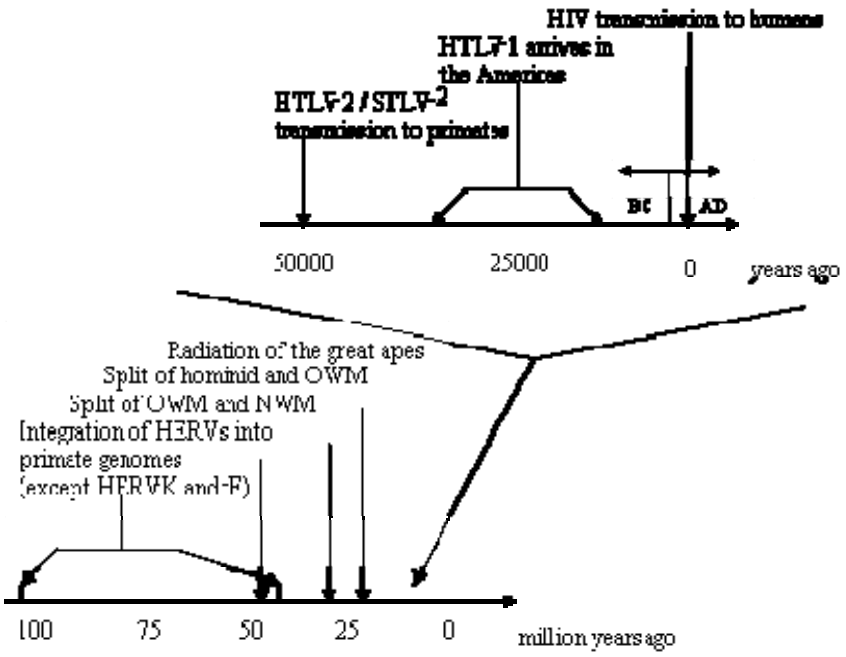


**Fig. 9: Retroviruses and the primate lineage.** The analysis of HERV elements in the primate lineage has shown that the majority of HERV sequences are present in all primates but prosimians (Blaise et al., 2003, Nelson et al., 2003, Salemi et al., 1999, Zhu et al., 1998, Goudsmit and Lukashov, 1999, Korber et al., 2000).

#### 1.3.6.1.2 Classification of endogenous retroviruses

Based on phylogenetic criteria, analysis of the human genome has led to the identification of at least 30 HERV-families (Benit et al., 2003). The first general approach was conducted on the *pol* gene (Tristem, 2000), which is the most conserved gene among retroviruses. It was then complemented by a survey performed on the *env* gene (Benit et al., 2001). Endogenous retroviral genomes probably all belong to the simple genera of retroviruses (Patience et al., 1997b). HERV families were found to be quite heterogeneous in numbers, from a few to a thousand elements, and in their degree of conservation, from 75% to more than 95% identity in the *pol* gene (Benit et al., 2003). HERVs have been grouped into three broad classes – I, II and III – on the basis of sequence similarity to different genera of infectious retroviruses. Each class has a number of subgroups, many of which are named according to an older system of HERV nomenclature based on the specificity of the tRNA primer binding site (PBS).

Class I HERVs are subdivided into six groups and are related to gammaretroviruses such as murine leukemia virus (MLV) and baboon endogenous virus (BaEV) in the highly conserved *pol* region and the *gag* and *env* regions. Class I includes HERV-W, HERV-H, HERV-I and HERV-R (ERV-9; Nelson et al., 2003) among many other subgroups Class II HERVs are subdivided into ten groups and related to betaretroviruses. They include mouse mammary tumor virus (MMTV) and several types of HERV-K elements

Class III HERVs are distantly related to spumaretroviruses and include HERV-L and HERV-S (Griffiths, 2001, Bannert and Kurth, 2004).

Class I and III HERVs are the oldest groups and are present throughout the primate lineage, while class II includes HERVs that have been active more recently. Many class II loci are restricted to chimpanzees and humans. A few proviruses of the HERV-K (HLM-2) subgroup are human-specific, indicating that these viruses have been active only within the last five million years (Griffiths, 2001). The rate of new human germ line insertions is presently at an extremely low level compared to earlier periods of evolutionary history or to the rate in some other mammals (Bannert and Kurth, 2004). At this time, only a small fraction of the youngest subtypes of Alu and L1 non-LTR-elements are still actively retrotransposing in humans (Medstrand et al., 2002). Yet it has been estimated that ~1 in every 100 to 200 human births has a *de novo* insertion of such a retroelement (Deininger and Batzer, 2002).

#### 1.3.6.1.3 Distribution of endogenous sequences within the human genome

The HERV regions are not uniformly distributed among the 2*22+2 chromosomes, but chromosomes 4, 19, X and Y have a higher density than expected from random distribution. The average is ~2.7 per Mb; the Y chromosome stands out with more than 14 HERVs per Mb and a 5 Mb window in chromosome Y (position 18-23 Mb) encompasses 120 HERV regions (Villesen et al., 2004, Kim et al., 2004).

#### 1.3.6.1.4 Biological implications of human endogenous retroviruses

Although HERVs have retained some similarity to their exogenous counterparts, they have acquired many mutations in the course of evolution. As a result, most HERV sequences are now defective or incomplete, with multiple stop codons, insertions, deletions, and frame shifts in the human genome (Lower et al., 1996, Wilkinson et al., 1994, Lee et al., 2000). Only a small fraction has escaped mutational decay. Conservation of an open reading frame during primate evolution clearly suggests some biological function and a minority of such sequences have acquired biological functions in humans. So far, only three HERV proviruses with complete ORFs for *gag*, *pol*, and *env* (the essential viral genes) have been identified, but at least one of these HERVs is mutated at a critical residue in the reverse transcriptase domain of *pol* (Griffiths, 2001). Loci from other HERVs have maintained a single intact open reading frame, such as the *env* genes from HERV-H (Lindeskog et al., 1999), HERV-W (Blond et al., 1999), and HERV-R (Cohen et al., 1988, Kim et al., 2004). The expression of HERVs can be influenced by different exogenous as well as endogenous agents (for a compilation see addendum 5.8). Some HERVs may be transcriptionally activated by hormones such as sex steroids and glucocorticoids (Ono et al., 1987). These hormonal effects are probably due to hormone-responsive elements in the LTRs. The structural genes of HERVs are preferentially expressed in the human placenta (Yi et al., 2004) and in several cancer cell lines (Armbruester et al., 2002), indicating that they may have biological and pathological roles. The consequences of de novo incorporation, reinfection, or retrotransposition of a retroelement can vary considerably. Pathologic,

irrelevant, and, in some cases, even beneficial outcomes have been reported for the expression of retroviral proteins and particles (Bannert and Kurth, 2004).

#### 1.3.6.1.4.1 Detrimental effects

Although RNA transcripts for multiple HERVs are expressed in normal tissues, the tissue-specific expression of HERVs has been associated with a number of chronic human diseases, including multiple sclerosis, diabetes, and rheumatoid arthritis (Perron and Seigneurin, 1999, Conrad et al., 1997, Nakagawa et al., 1997, Murphy et al., 1998, Lower et al., 1998, Lan et al., 1998). Though it is still unclear whether the expression of endogenous sequences is involved in disease development or whether it is an epiphenomenon, different disease mechanisms are proposed. These include a role in methylation for HERV-E sequences (Okada et al., 2002) and the suggestion that expression of syncytin in astrocytes induces the release of redox reagents which are cytotoxic to oligodendrocytes (Antony et al., 2004).

Besides AIDs, expression of HERVs has frequently been linked with various cancers (Lower, 1999, Armbruester et al., 2002, Patience et al., 1997b), for example, retrovirus particle formation in teratocarcinoma cell lines (Lower et al., 1996). An association of endogenous sequences with the onset of schizophrenia has also been suggested (Karlsson et al., 2001).

Conversely, the absence of HERV expression has also been linked to cancer (Kato et al., 1988).

#### 1.3.6.1.4.2 Advantageous effects

The best example of an HERV with a known biological function is HERV-W. An envelope protein derived from this HERV – syncytin – mediates the fusion of trophoblasts, an essential step during formation of the placenta (Mi et al., 2000, Blond et al., 1999). Fusion was also observed for the envelope of HERV-FRD (syncytin 2). A search for the FRD*env* gene in primates indicated that the corresponding proviral element is present in all simians, from New World monkeys (NWM) to humans, being absent only in prosimians (Blaise et al., 2003). A role in membrane fusion is not surprising, since fusion is mediated by the viral Env protein during retroviral infection following binding to a cell surface receptor (see replication cycle, fig. 8). HERV-R (ERV-3) is also highly expressed in trophoblastic cells and results in high concentrations of Env protein in syncytiotrophoblasts. The immunosuppressive potential of this HERV and the fusogenic nature of placenta tissue suggest a possible involvement in normal placenta formation, the protection of the developing fetus from maternal immune responses (Boyd et al., 1993, Venables et al., 1995).

Another function proposed for HERVs is determining resistance to viral infection by interference mechanisms. Mechanisms conferring protection against a related viral agent may include retroviral receptor blockade (by HERV products) and interference of replication by antisense mRNA (Griffiths, 2001, Nelson et al., 2003). The destruction of

lymphocytes during lytic retrovirus infections as well as envelope gene derived immunosuppressive retroviral gene products may prevent chronic inflammatory diseases and tissue destruction (Hermann et al., 1998).

#### 1.3.6.1.5 Multiple sclerosis associated endogenous retroviruses

Expression of retroviral sequences and antibodies directed against retroviral proteins has been described repeatedly. The families of the endogenous retroviruses currently in the focus of research interest – the HERV-H related RGH2 and the HERV-W related multiple sclerosis associated retrovirus (MSRV) – contain an ancestral proviral copy on chromosome 7 at about 1 kb distance from one another. This region is one of two genetic loci previously identified as potentially associated with 'multigenic' susceptibility to MS (Perron et al., 2000).

##### 1.3.6.1.5.1 HERV-H/RGH

HERV-H sequences with high homology to the HERV-H variant RGH2 were found specifically at particle level in cell-free plasma from 24 of 33 Danish MS patients, but could not be demonstrated in 29 plasma samples from patients with other diseases nor in 20 plasma samples from healthy controls. In gradient preparations of patient samples, reverse transcriptase activity and amplifiable retroviral sequences were detected in fractions that are typical for retrovirus banding. Christensen et al. suggested that MS is associated with replication of otherwise quiescent endogenous retroviruses (Christensen et al., 2000). It was shown that HERV-H particles derived from cell cultures of MS patients were transmissible, albeit at a very low level (Christensen et al., 2002).
The HERV-H / RTLV-H family members are present as multicopies in the human genome, 800 to 1000 copies and additionally ~ 1000 solitary LTRs are found in all chromosomes with concentration on chromosomes 1p and 7q (Nelson et al., 2004). Different RTLV-LTRs which are integrated in the human genome were described to act as promoter and polyadenylation signal for several genes (Brosius, 1999).

##### 1.3.6.1.5.2 MSRV/HERV-W

In France a HERV-W related retrovirus, termed the multiple sclerosis associated retrovirus (MSRV), was described for the first time in (Perron et al., 1989). MSRV sequences were detected in controls without MS (7%), in MS patients (53%), but in 100% of patients who were untreated at the time of sampling (Garson et al., 1998). Specific reverse transcriptase activity was detected in 12 out of 21 cultures from patients with MS (Perron et al., 1991). Retroviral particles were produced by cell cultures from patients with MS (Perron et al., 1997, Blond et al., 1999, Komurian-Pradel et al., 1999).
Sardinia has one of the highest MS prevalence and incidence rates worldwide; the incidence rate tripled from the 1960s to the mid-1990s. A 100% correlation between evidence of extra cellular MSRV in the plasma and the presence of MS was described, with 12% of healthy blood donors harboring the virus (Serra et al., 2001).

Cloning of a replication competent MSRV provirus has not been successful (Perron and Seigneurin, 1999). MSRV in the CSF may have gliotoxic and superantigenic properties and could be associated with more disabling MS (Sotgiu et al., 2002, Serra et al., 2003). HERV-W/MSRV is a multicopy virus, the human genome contains at least 70, 100, and 30 HERV-W related *gag*, *pro*, and *env* regions, respectively (Nelson et al., 2004). The complete nucleotide and protein sequences of MSRV/HERV-W have been characterized and the provirus has been localized on chromosome 7q (Voisset et al., 2000, Alliel et al., 2002). Proteins of known amino acid sequences expressed from the *pol*-segment showed 30% to 65% homology to virus proteins from herpes simplex virus and feline sarcoma virus (Alliel et al., 2002). Syncytin, a protein that is encoded by the HERV-W*env* gene, is expressed at a rate three times higher in the brains of MS patients than in the brains of healthy people. Moreover, syncytin is expressed selectively in astrocytes and microglia, cells that mediate neuroinflammation (Antony et al., 2004). One of the HERV-W proviral sequences is located within a TCR alpha-delta receptor gene in chromosome 14q, one of the two genetic loci previously identified as potentially associated with 'multigenic' susceptibility to MS (Perron et al., 2000).

#### 1.3.6.1.6 Endogenous animal retroviruses

Several endogenous retroviruses in various animals are able to produce particles. MMTV and murine leukemia virus in mice (Griffiths, 2001, Coffin et al., 1983), Jaagsiekte retrovirus (JSRV) in sheep (Boeke and Stoye, 1997), porcine endogenous retroviruses (PERVs) in pigs (Wilson et al., 1998; Patience et al., 1997a), avian leukemia viruses (AVL) in chickens, SMRV in NWM (Schochetman et al., 1977), baboon endogenous retrovirus (BaEV) in baboons and their close relatives (Todaro et al., 1976), as well as feline leukemia virus in cats have presently both endogenous and exogenous strains (Boeke and Stoye, 1997, Rasmussen, 1997). It was observed that the endogenous JSRV blocks the entry of the corresponding exogenous virus (Spencer et al., 2003).

### 1.3.7 Exogenous human retroviruses

In 1971 the first retrovirus was isolated from a human cell culture. It was called human spumaretrovirus (HSRV) or human foamy virus (HFV; Achong et al., 1971). Although these viruses caused syncytia formation in cell culture, they could not be linked to any disease in humans or animals. Today it is assumed that there is no 'human' foamy virus, but zoonotically transmitted viruses which are detected in cell culture and some individuals, mainly persons with close contact to infected monkeys. Four human retroviruses are currently known: the human T cell lymphotropic virus types I and II (HTLV-1/2) and the human immunodeficiency virus types 1 and 2 (HIV-1/2).

#### 1.3.7.1 Human T cell lymphotropic virus (HTLV)

HTLV-1 was the first human pathogenic retrovirus to be identified. It was isolated from a patient with cutaneous T cell lymphoma (Poeisz et al., 1980, Reitz et al., 1981). HTLV-1,

which shares 60% sequence identity with HTLV-1, was isolated from a variant form of hairy T-cell leukemia cells (Kalyanaraman et al., 1982). HTLV-1 and HTLV-2 are ancient viruses that have migrated with their hosts between Africa, Asia, Austronesia and the Americas. Phylogenetic analyses suggest that HTLV-2 infection crossed the Bering Strait with its human host 10,000 to 35,000 years ago, while the Melanesian HTLV-1 subtype was probably transmitted from Indonesian macaques to humans 50,000 years ago (Salemi et al., 1999).

#### 1.3.7.1.1 Epidemiology of HTLV infections

Today the recognized endemic areas for HTLV-1 are Japan, Africa, Melanesia, South America, the Caribbean and the southern states of the USA (Taylor, 1999). HTLV-2 seems to be geographically restricted; initial reports of endemicity were restricted to isolated Native American populations (Hall et al., 1996), and it is now described to be endemic in Amerindians of North, Central and South America, in several Pygmy tribes across Central Africa (Goubau et al., 1993), and possibly in Central Asia (Hall et al., 1994). Seroprevalence in endemic areas is 1% to 5%, although clusters are found in which the prevalence is much higher, e.g. southern Japan up to 30%. HTLV infections and related diseases have been reported not only in the tropics but also among immigrants in Europe and the USA (Cruiskshank et al., 1989) and need to be differentiated from MS (Poser et al., 1990) and other diseases (syphilis, subacute combined degeneration and Behçet's disease, tropical diseases). In Germany only sporadic cases have been described, usually related to migrants from endemic areas (Fleischer et al., 1999).

#### 1.3.7.1.2 Transmission of HTLV

HTLV can be transmitted intrauterine or via breast milk from mother to child (vertical transmission, Hino, 1989), horizontally by sexual intercourse, more frequently from male to female than vice versa (Kajiyama et al., 1986), and by transfusion of blood components (Osame et al., 1990) or needle sharing (Schwebke et al., 1994).

#### 1.3.7.1.3 Diagnosis of HTLV infection

Diagnosis of HTLV infection can be accomplished by serological tests (Western Blot, ELISA) and detection of the virus in cell culture, electron microscopy, or detection of virus nucleic acid.

#### 1.3.7.1.4 Pathology of HTLV infections

Adult T cell leukemia (ATL) and HTLV associated myelopathy/tropical spastic paraparesis (HAM/TSP) are caused by HTLV-1, albeit at a low frequency of approximately 1% of HTLV-1 carriers. Several other neurological syndromes have been linked to HTLV-1 infection (Osame et al., 1990*),* and more than 60 different diseases and syndromes have been suggested to be linked to this retrovirus infection (Gessain et al., 1985, Rodgers-Johnson et al., 1985, Roman and Osame, 1988, Zaninovic, 2004). The latent virus infection

persists in the T cells of infected individuals, including those who remain asymptomatic. Antibodies also persist throughout. Increased numbers of autoreactive T cells have been reported in patients with HTLV-1-associated myelopathy (Usuku et al., 1988). Evidence that HTLV-2 is also associated with a chronic myelopathy is now accumulating (Hall et al., 1996).

#### 1.3.7.1.5 Treatment of HTLV associated diseases

No curative therapy is currently available. Chemotherapy applied to other leukemias has turned out to be of rather short term effect; the same was observed for interferon alpha or gamma therapy. Inhibitors of protease used for anti HIV therapy are not effective for HTLV reverse transcriptase, and results obtained with inhibitors of reverse transcriptase are also not encouraging (Gürtler, 2002).

### 1.3.7.2 Human immunodeficiency virus HIV

The clinical picture of the acquired immunodeficiency syndrome (AIDS) was first described in 1981 (Masur et al, 1981). Two years later, retrovirus particles were isolated from the lymph nodes of a patient (Barré-Sinoussi et al., 1983, Chermann et al., 1983). This retrovirus was classified as a lentivirus and identified as the causative agent for the observed ‘acquired immunodeficiency syndrome’ by the groups of Luc Montaigner (Barré-Sinoussi et al., 1983 and Robert Gallo (Popovic et al., 1984). A second HIV-type (HIV-2) was described in 1986 (Starcich et al., 1986).

The oldest known sample with detected antibodies against HIV dates back to 1959 (a serum from Congo-Zaire; Zhu et al., 1998). The zoonotical transmission of the virus from non-human primates to humans was estimated to have occurred between 1912 and 1950, depending on the phylogenetic model and genome region (Zhu et al., 1998, Goudsmit and Lukashov, 1999, Korber et al., 2000).

#### 1.3.7.2.1 Epidemiology of HIV infections

Although HIV has been known only since the early 1980s, it has become a major global public health problem (addendum 5.9). At the end of 2004 more than 40 million people were infected with HIV worldwide (UNAIDS; addendum 5.10), and at the end of 2004 about 44,000 people were infected in Germany (addendum 5.11). The incidence rate in Germany is approximately 2000/year (www.rki.de, 2005).

#### 1.3.7.2.2 Classification of HIV

HIV-1 and HIV-2 are distinguished from each other based on molecular and biological differences. Although both cause immunodeficiencies and share similar morphological structures, HIV-1 predominates worldwide (Coffin et al., 1995, Ho, 1995). HIV-1 is subdivided into three groups, M (main), N (near M) and O (outlier). Based on sequence variations, M is further differentiated into ten subtypes A to K. HIV-2 can be subdivided into subtypes A to F (Gürtler, 2002).

#### 1.3.7.2.3 Transmission of HIV

HIV is transmitted horizontally by sexual contact, blood or blood products, and vertically from an infected mother to infant, either intrapartum, perinatally, or via breast milk (Gürtler, 2002).

#### 1.3.7.2.4 Pathology of HIV infections

The primary cellular targets of HIV in infected humans are CD4+ cells like macrophages and T helper cells. In untreated individuals, the continuing replication of virus results in a steady decline in the number of CD4+ T cells. In the course of time the immune system is overwhelmed, with the result that the replication of HIV escalates, opportunistic fungal, protozoal, bacterial, and viral infections occur, characteristic cancers appear, and neurological symptoms become apparent. The clinical course has four stages: (1) primary infection, (2) asymptomatic infection, (3) persistent generalized lymphadenotpathy, and (4) symptomatic stage, termed the acquired immunodeficiency syndrome (AIDS; Gürtler, 2002).

#### 1.3.7.2.5 Diagnosis of HIV infection

Diagnosis of HIV infection can be accomplished by serological tests (Western Blot, ELISA) and detection of the virus in cell culture, p24 antigen capture assay, electron microscopy, or detection of virus nucleic acid.

#### 1.3.7.2.6 Treatment of HIV infection

In 1996 the ‘highly active antiretroviral therapy’ (HAART) was introduced, consisting of combinations of nucleoside and non-nucleoside reverse transcriptase inhibitors, and protease inhibitors. Opportunistic infections and tumors are treated with antimicrobial drugs and chemotherapy as appropriate (Gürtler, 2002).

## 1.4 Aims

Findings and knowledge about the clinical pictures caused by known human and animal retroviruses indicate a possible involvement in the etiopathogenesis of AIDs. The aim of this work was to establish sensitive retrovirus-specific as well as sensitive retrovirus-generic methods that could be applied to cell cultures as well as to patient samples, such as whole blood or cerebrospinal fluid (CSF). Following evaluation of the generic assays, using retrovirus infected cell cultures and retrovirus-positive plasma, the assays were then to be applied to blood samples from patients. Blood samples from MS patients were to be tested quantitatively for the expression of MS associated retrovirus sequences and HTLV.

### 1.4.1 Samples

Blood samples from patients with three different rheumatic disorders, namely SLE, scleroderma, and spondylitis ankylosans were to be analyzed, using the adapted and optimized generic methods in order to assess the presence of any retroviruses.

Two cell cultures (MS1533 and MS1845) had previously been described to show RT activity and a transmissible retroviral agent (Christensen et al., 2000, Christensen et al., 2002). These cell lines were to be analyzed and if they were found positive for the described HERV-H expression, the virus genome was to be sequenced by genome walking. German MS patients had not yet been analyzed for specific expression of HERV sequences. Three different groups of MS patients were to be analyzed in comparison to healthy controls: (1) samples from children and adolescents with EOMS in remission for the last six months or (2) active EOMS, and (3) samples from adult MS patients. Furthermore, children and adolescents with inflammatory and non-inflammatory neurological diseases were to be used as a diseased control group. Samples from patients with SLE, scleroderma or spondylitis ankylosans were to be analyzed as diseased adult control groups.

### 1.4.2 Generic approaches

Two different methods were considered. The differential display (DD) approach, a subtractive technique, relying on different expression patterns in infected and non-infected individuals, was adapted as a generic screening method beforehand (Uhlenhaut, 2000). The DD approach using specific primers allows a pre-sorting of sequences and thus a simplification of the original assay. A short genome region similar for all retroviruses was to be used to define a small number of target sequences. The DD was to be established using cell culture supernatants. The transfer of the method to blood samples from patients in comparison to healthy controls was to be established. This method was intended not only to detect ‘new’ or zoonotically transmitted viruses, but also to be able to reflect abnormal expression patterns caused by aberrant expression of endogenous retrovirus sequences.

The second retrovirus-specific method is the reverse transcriptase activity assay (Pyra et al., 1994, Boni et al., 1996). Although this assay has been described as extremely sensitive, it is based on magnesium as necessary cation only. Reverse transcriptase (RT) enzymes could use magnesium or manganese with different efficiencies, thus the sole use of $Mg^{2+}$ could leave $Mn^{2+}$-preferring RTs undetected. The reverse transcriptase activity assay was to be evaluated, if necessary optimized, and the $Mn^{2+}$ testing to be developed.

### 1.4.3 Exogenous retroviruses – specific approach

Expression of the HTLV*tax* sequence or antibodies directed against HTLV*tax* has been described in many AIDs, although only in a fraction of patients and sometimes in healthy controls as well. Real-time PCR was to be used in order to analyze all samples for the presence of this HTLV sequence.

### 1.4.4 Endogenous retroviruses – specific approach

In contrast to potentially undetected ‘new’ exogenous retroviruses, sequence information about endogenous retroviruses is available. The difference between a healthy and diseased state may be characterized by different expression levels. Since endogenous retroviruses may be newly acquired (e.g. zoonotically), or expression may rise with time (immunosenescence), the goals included the comparison of expression levels not only for different health status but also for different ages. Expression levels of different endogenous retrovirus sequences were to be analyzed quantitatively by real-time PCR.