Preface

"...we simply do not know enough, we are still a largely ignorant profession, faced by an array of illnesses which we do not really understand, unable to do much beyond trying to make the right diagnosis, shoring things up whenever we can by one halfway technology or another..."

Lewis Thomas,
The Fragile Species, TOUCHSTONE*, New York, 1992*

Principles of Molecular Rheumatology has been organized to help Rheumatology Fellows, House Officers, and Rheumatologists better understand the molecular and cellular aspects of Rheumatic Diseases. The ambition of the editor and the authors is to present and discuss the pathogenesis of rheumatic diseases in a concise manner. We hope that *Principles of Molecular Rheumatology* will facilitate the introduction of clinical trainees to the science of Rheumatology and will serve as a helpful accessory in reviewing basic and clinical articles with reference to basic science issues. Furthermore, it is our intention to help those students of human disease who do not have a formal medical training gain an informed perspective on rheumatic diseases.

The first section of *Principles of Molecular Rheumatology* discusses the molecular mechanisms that are central to many rheumatic diseases. Established authors present the biochemical mechanisms by which apoptosis, cell signaling, complement, lipids, and viruses contribute to disease expression. The second section reviews immune and nonimmune cell function as it relates to rheumatic diseases. The function of lymphocytes, monocytes, neutrophils, synoviocytes, chondrocytes, and bone cells is discussed. The third section takes a synthetic approach to disease. The authors present integrated discussions of the cellular, biochemical, and molecular biological mechanisms that are directly important to disease pathogenesis. Major diseases are reviewed and concepts are formulated. In the final section, the molecular aspects of those therapeutics that are routinely used in rheumatic diseases are discussed. The emphasis on mechanisms rather than clinical pharmacology aims at familiarizing the reader with what is being accomplished at the molecular and cellular levels following the administration of each medication.

Principles of Molecular Rheumatology does not replace any of the classic textbooks in Rheumatology. Rather, it adopts a fresh perspective designed to enhance the understanding of Rheumatology by emphasizing the importance of knowledge of molecular and cellular pathophysiology to the mastery of rheumatic diseases.

I am grateful to the authors for many exciting discussions on the format and content of the book and for their enthusiasm and support, which provided me with the stamina to see the project to its completion. I learned so much from my interactions with my esteemed colleagues, authors of *Principles of Molecular Rheumatology*, that I do not seek reward. My only hope is that *Principles of Molecular Rheumatology* will help our fellow Rheumatologists better serve the patients who suffer from rheumatic diseases. The unwavering support of Paul Dolgert is once more appreciated. Craig Adams and Elyse O'Grady are responsible for all the good things in this book, whereas I am responsible for its shortcomings

George C. Tsokos, **MD**

2

Viruses

Andras Perl

1. Introduction

Viruses are considered as key environmental factors that may cause inflammatory arthritis and autoimmune diseases in genetically susceptible hosts. Viruses can elicit acute or subacute and, less often, chronic forms of arthritis. These viral arthritis syndromes can be diagnosed by recognition of well-defined clinical signs and detection of viral antibodies and nucleic acids. Moreover, viral elements may also play a role in the pathogenesis of idiopathic autoimmune rheumatic diseases. The concordance rate of the most common autoimmune disease, such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), in monozygotic twins is about 25% *(1)*. Although these data show that genetic factors influence susceptibility to autoimmune diseases, alternatively, a 70% discordance rate emphasizes the importance of environmental factors. Forensic studies of archeological sites revealed the presence of RA-like erosive bony changes in pre-Columbian New World populations dating back 6500 yr and the absence of RA in the Old World before the 18th century *(2)*. This geographic distribution suggest that RA may have spread from the Americas through environmental factors, possibly by a virus, another microorganism, or an antigen. The potential etiological role of viruses in chronic rheumatic diseases have been recently reviewed *(3)*. This chapter will focus on known viruses capable of causing rheumatic diseases, review molecular techniques suitable to identify viruses, describe the latest experimental evidence implicating known and unidentified viruses in the causation of idiopathic autoimmune rheumatic diseases, and review mechanisms of viral pathogenesis, molecular mimicry, and altered apoptosis that can result in autoimmunity.

2. Virus-Induced Arthritis Syndromes

Viral infections are often associated with arthralgias, whereas arthritis occurs less commonly. Most cases of virus-induced arthritis are short-lived and self-limited as a result of an efficient elimination of the organism by the immune system. Examples of these viral syndromes include rubella and parvovirus B19-induced arthritis. Chronic joint diseases have been associated with persistent or latent viral infections, largely the result of an inability of the immune system to eliminate the pathogen, virus-induced autoimmunity, characterized by molecular mimicry, polyclonal B-cell activation, and immunodeficiency resulting in opportunistic infections. This latter group of pathogens

From: *Current Molecular Medicine: Principles of Molecular Rheumatology*
Edited by: G. C. Tsokos © Humana Press Inc., Totowa, NJ

include human immunodeficiency virus 1 (HIV-1), human T-cell lymphotropic virus type I (HTLV-I), and hepatitis C virus (HCV).

2.1. Virus-Induced Transient Arthritis Syndromes

2.1.1. Parvovirus B19

This small single-stranded DNA virus is one of the most frequent causes of viral arthritis in humans *(4)*. The majority of parvovirus B19 infections occur in children 5–15 yr of age. The virus spreads through respiratory or oral secretions. After a 1- to 4-wk incubation period, most children present with erythema infectiosum with a typical facial rash (slapped cheeks), headache, low-grade fever, coryza, cough, conjunctivitis, and/or mild gastrointestinal symptoms. Whereas arthritis occurs in 5% of childhood infections, 50% of adults freshly infected by the virus develop joint pain and swelling (Table 1). Joint pain develops with equal frequency in boys and girls. In the adults, arthritis is more frequent in females, and skin eruptions are far less conspicuous. The virus can infect bone marrow cells, preferentially erythroid progenitors, thus causing anemia, neutropenia, and thrombocytopenia with transient aplastic crises. Vasculitic peripheral neuropathies and liver disease with elevated transaminases have been also reported. Parvovirus B19 arthritis usually presents with acute, moderately severe, symmetric polyarthritis first affecting the hands and knees, then rapidly spreading to the feet, elbows, and shoulders. Joint manifestations are temporally associated with production of anti-B19 IgM antibodies. These antibodies clear the viremia, thus rendering the patient noninfectious. IgM antibodies are present for up to 3 mo after infection. Whereas IgM antibodies are diagnostic for a recent B19 infection, IgG antibodies are long-lived and not useful for diagnosis. Seroprevalence of IgG antibodies may be as high as 80% in the adult population. Joint pain and synovitis usually lasts for 2–8 wk and rarely persists for more than 3 mo. Patients have morning stiffness that can last for more than 1 h, symmetrical swelling of the wrist and metacarpophalangeal and proximal interphalangeal joints. Patients may have low-titer rheumatoid factors. Failure to recognize sometimes minimal skin eruptions and obtain B19 IgM antibody titers may lead to a diagnosis of early RA. However, erosions and joint destructions have not been described in B19 arthropathy. Whereas involvement of B19 has been repeatedly raised in classic RA *(5)*, large surveys failed to demonstrate an association between erosive RA and parvovirus B19 *(4)*. B19-Specific DNA can be detected in synovium and synovial fluid of patients with chronic B19 arthropathy. B19 DNA was also detected in normal noninflammatory synovium as well *(6)*. It is presently unclear whether intraarticular viral infection is prerequisite of B19 arthropathy.

2.1.2. Rubella Virus

Rubella virus has a single-stranded RNA genome. Viral infection is a mild and self-limited disease characterized by skin rash, lymphadenopathy, and low-grade fever. Infection may also lead to subclinical illness. Infection during pregnancy can result in fetal malformations. Rubella is known to cause arthralgias and acute arthritis in one-third of patients after both natural infection and vaccination *(7)*. Similar to parvovirus B19, joint symptoms are more common in women than in men or children. Rubella arthritis affects the small joints of the hands, wrist, elbows, or knees and rarely lasts more than 1 wk. Chronic arthropathy was reported in 1–4% of postpartum female

Table 1
Arthritis in Viral Infections

Virus	Genome	Arthritis frequency	Arthritis type	Duration	Erosion	Diagnosis	Ref.
HCV	RNA	10–50%	Polyarticular, symmetrical	Chronic	No	ELISA, WB[a], PCR	20,21
HBV	DNA	10–25%	Symmetrical, migratory	1–3 wk	No	ELISA, WB	3
Parvovirus B19	DNA	Children: 5–10% Adults: 50–70% Female : male = 2 : 1	Polyarticular, small and large joints, symmetrical	2–8 wk	No	ELISA	4
Rubella	RNA	10–30%	Multiple small joints	5–10 d	No	ELISA, WB, PCR	3,7
VZV	DNA	<1%	Monoarthritis	1–7 d	No	ELISA, WB	18
EBV	DNA	1–5%	Poly- or monoarthritis	1–12 wk	No	ELISA, WB	110
HSV-1	DNA	Case reports	Monoarthritis	1–10 d	No	ELISA	111,112
HTLV-I	Retrovirus	<1%	Oligoarthritis, large joints	Chronic	Yes	ELISA, WB, PCR	30,113
HIV-1	Retrovirus	10–50%	Painful joint syndrome Reiter's syndrome Psoriatic arthritis	1–2 d Chronic Chronic	No Yes Yes	ELISA, WB, PCR	13

[a]WB = Western Blot analysis.

recipients of the RA27/3 vaccine strain *(8)*. Other studies found no increase of chronic arthritis in women receiving the RA27/3 rubella vaccine *(9)*. No rubella virus can be recovered from peripheral blood lymphocytes of persons with chronic arthropathy following rubella infection or vaccination *(10)*. Neuropathic syndromes, such as carpal tunnel syndrome, does not appear to have an increased rate among recipients of the RA27/3 vaccine *(9)*. These data support the continued vaccination of rubella-susceptible females to reduce the risk of congenital malformations.

The rubella vaccine is part of a combined measles–mumps–rubella preparation. Arthritis is not uncommon in natural mumps infection, especially in adult man. Arthritis occurs 1–3 wk after the onset of parotitis and may be associated with low-grade fever. Mumps can cause migrating polyarthritis, monoarthritis of the knee, hip, or ankle, or arthralgias alone. Mumps arthritis is self-limited, rarely lasting more than 4 wk *(11)*. Measles have not been associated with joint symptoms. A recent survey failed to show evidence for involvement of measles, mumps, or rubella virus in chronic arthritis *(12)*.

2.1.3. Herpesviruses

Members of the Herpesviridae family have a large (> 100 kb) double-stranded DNA genome. After initial infection, herpesviruses persist in the host with lifelong latency. Therefore, several of these viruses have been considered as etiologic agents in autoimmune diseases, such as SLE, RA, or Sjogren's syndrome (*see* Subheading 4.).

The cytomegalovirus (CMV) infects most individuals during their life. Major syndromes associated with CMV include inclusion disease in neonates, heterophile antibody-negative mononucleosis in healthy individuals, and pneumonitis, arthritis, vasculitis, and chorioretinitis in immunocompromised individuals *(13)*.

The Epstein–Barr virus (EBV) commonly causes subclinical infections of heterophil antibody-positive mononucleosis in young adults. Arthralgias lasting for up to 4 mo occur in 2% of patients with mononucleosis. Recently, EBV-positive lymphomas were described in methotrexate-treated RA patients *(14–17)*. Interestingly, remission of lymphomas was noted after discontinuation of methotrexate *(14,15)*.

Herpes simplex virus 1 (HSV-1) can cause monoarthritis few days after the onset of oral or genital lesions. HSV-1 arthritis rarely last longer than 2 wk. Varicella–zoster virus (VZV) can cause monarthritis, mostly in the knee, as a rare complication of chickenpox *(18)*. HZV causes shingles in the elderly or immunosuppressed host after reactivation from dorsal root ganglia.

2.1.4. Hepatitis B Virus

Arthralgias and arthritis occur early after hepatitis B virus (HBV) infection. Arthritis is characterized by a sudden onset, symmetrical polyarticular synovitis of the small joints of the hands and knees, erythematous and pruritic rash, anorexia, malaise, and fever. Arthritis resolves in 2–6 wk with the onset of jaundice. Hepatitis B virus has been also associated with polyarteritis nodosa and cryoglobulinemia. Erythema nodosum, uveitis, and polyarthritis were rarely reported following immunization with recombinant HBV vaccine.

2.2. Virus-Induced Chronic Arthritis

2.2.1. Hepatitis C Virus

Hepatitis C virus (HCV) is a single-stranded RNA virus. Based on genomic variability, at least six subtypes have been identified. The virus is transmitted parenterally,

primarily through the exchange of body fluids. Viral infection can be detected by PCR within 2 wk of exposure. Serum transaminases and antibody titers become elevated after 4–8 wk. Despite high-titer antibody levels, >80% of infected individuals become chronic virus carriers. HCV has a wide pathogenic potential that is not limited to diseases of the liver, chronic active hepatitis, cirrhosis, and hepatocellular carcinoma *(19)*. Identification of HCV as the cause of most cases (>90%) of type II or essential mixed cryoglobulinemia (EMC) is a major breakthrough in rheumatology *(20)*. Type II cryoglobulins are immune complexes comprised of a monoclonal IgM/kappa rheumatoid factor and polyclonal IgG. HCV-RNA is concentrated 1000-fold in the cryoprecipitate in comparison to the serum *(21)*. The clinical syndrome of EMC is an immune-complex vasculitis characterized by purpura, arthralgias, inflammatory arthritis, peripheral neuropathy, and glomerulonephritis *(22)*. IgM/kappa-bearing B cells are clonally expanded in the peripheral blood of EMC patients *(23)*. Infection by HCV may be directly responsible for the clonal expansion of B cells *(24)*. This process may lead to development of B-cell non-Hodgkin's lymphomas *(19)*.

The HCV infection is associated with production of autoantibodies. Up to 75% of the patients have high-titer rheumatoid factors, presumably produced by HCV-infected and thus clonally expanded B-lymphocytes. Half or more of the patients have anti-smooth-muscle antibodies. Low-titer antinuclear antibodies and anticardiolipin antibodies were noted in 10–30% of HCV-infected patients. Five percent of patients may develop Sjogren's syndrome. SLE, autoimmune thyroiditis, erosive/rheumatoid arthritis, and polymyositis/dermatomyositis were rarely documented *(19)*.

Cryoglobulinemia is detectable in 40–50% of HCV-infected patients *(19,25)*. Treatment with interferon-α and ribavirin appears to be effective in reducing viral RNA and cryoglobulin levels. Unfortunately, only 20% or less of the patients will have a sustained remission after discontinuation of antiviral therapies.

2.2.2. Human T-Cell Lymphotropic Virus I (HTLV-I)

Infection by human T-cell lymphotropic virus I (HTLV-I) has been associated with adult T-cell leukemia (ATL), mycosis fungoides/Sézary syndrome, HTLV-I-associated myelopathy/tropic spastic paraparesis (HAM/TSP), HTLV-I associated arthritis (HAA), polymyositis, and Sjögren's syndrome *(26)*. HTLV-I infection occurs in endemic areas of southwest Japan, the Caribbean basin, the southeastern United States, and parts of Africa. Despite very high rates of infection in endemic areas where 30% or more of the population may be infected, relative few (<1%) infected individuals show disease manifestations attributable to HTLV-I. The lifetime risk of developing a HTLV-I-associated disorder is less than 5%. The vast majority of virus carriers remain disease-free and serve as a huge reservoir for further transmission of the virus. The virus spreads through three major routes: from mother to child via breast-feeding, sexual intercourse, and contaminated blood products via transfusion or intravenous drug use. ATL usually presents in middle-aged adults in the forms of acute high-grade leukemia with widespread systemic involvement resulting from infiltration of the skin, liver, spleen, lungs, lymph nodes, bone marrow, salivary glands, and/or synovium. A chronic cutaneous involvement is characterized by leukemic cell infiltration of the dermis and subcutaneous tissue. Polymyositis, Sjögren's syndrome, and inflammatory arthritis may occur in the absence of leukemia. These latter conditions clinically are

indistinguishable from the idiopathic autoimmune syndromes. They are characterized by infiltration of the skeletal muscle, salivary glands, or synovium with HTLV-I infected T-lymphocytes. T-cells infiltrating the joint have indented cerebriform nuclei similar to those seen in ATL. HAA patients develop chronic oligoarthritis, primarily affecting the larger joints (knees and shoulders). Patients may have rheumatoid factors and X-ray films show joint-space narrowing with erosions. HTLV-I infection can be diagnosed with enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot. PCR techniques are more sensitive that serological assays and allow the differentiation between HTLV-I and a less common virus, HTLV-II. Clinical significance of HTLV-II infection, most often found among intravenous drug abusers in the United States, is presently unknown. Transgenic mice carrying the *tax* transactivator gene of HTLV-I develop Sjögren's syndrome and rheumatoid-like arthritis *(26)*. These studies provided experimental evidence for a pathogenic role of the HTLV-I p40/tax protein. Prevalence of RA is increased in the HTLV-I-infected population (0.56%) with respect to the uninfected population of Japan (0.31%) *(26)*. Thus, the relatively low disease frequency in virus-infected individuals strongly advocates for the role of factors other than HTLV-I in the development of RA.

2.2.3. Human Immunodeficiency Virus 1

In the United States, more than 1 million individuals are infected by human immunodeficiency virus 1 (HIV-1). HIV-1 enters cells by fusion at the cell surface, triggered by binding of the gp120 envelop protein to the CD4 molecule of the host cell. A second receptor for HIV was recently identified as the receptor for β-chemokines *(27)*. Interestingly, homozygous defects in the β-chemokine/HIV-1 coreceptors CCR2 and CCR5 appear to be responsible for resistance of some individuals (<10%) to HIV-1 infection. These new findings may provide new means in preventing or slowing HIV disease. During the course of HIV-1 infection, three major phases can be distinguished. Within a few weeks after infection, extensive viremia occurs, giving rise to an acute mononucleosis-like syndrome. This period is characterized by flu-like symptoms, arthralgias, and lymphadenopathy, accompanied by a robust activation of the immune system. When humoral and cellular immune responses to HIV become established, a subclinical phase of disease with relatively minor changes in CD4 T-cell counts ensues. Recently, however, it became clear that this second or latent period represents an ongoing fierce battle between virus replication and replenishing of the CD4 T-cell reservoir. On the average, 10 yr following infection, virus-infected cells and viral RNA levels drastically increase with a sharp decline of CD4 T-cell counts in the peripheral blood. Both direct infection of various cell types and tissues and secondary changes in the lymphokine milieu are important for the pathogenesis of HIV disease. Disease progression has been attributed to a shift from Th1-type to Th2-type helper-T-cell predominance resulting in polyclonal B-cell activation, hypergammaglobulinemia, and production of autoantibodies. Rapid decline of CD4 T-cell counts is mediated by increased apoptosis sensitivity of HIV-infected cells. Diminished CD4 T-cell function gives rise to opportunistic infections, lymphomagenesis, and autoimmune phenomena at the final stages of disease. Autoimmune rheumatic diseases most commonly noted in patients with autoimmunodeficiency syndrome (AIDS) include Reiters syndrome, psoriatic arthritis, spondylarthropathies, and diffuse infiltrative lymphocytosis syndrome (DILS). Inter-

estingly, all of these syndromes have been associated with relative expansion of CD8 T-cells, thus suggesting that HIV-1 infection accelerates HLA class I-restricted CD8 T-cell-mediated autoreactivity *(28)*. In turn, SLE, RA, and polymyositis, which are thought to be mediated by CD4 T-cells, remit in some patients following infection by HIV-1 *(13)*.

3. Molecular Techniques for Detection of Viruses

Diagnosis of viral infections can be made by serologic testing of virus-specific antibodies or detection of viral nucleic acids. ELISA or radioimmunoassays are best suited for rapid screening of antibody reactivities *(29)*. Immunoreactivities to viral proteins generally require confirmation by Western blot. In comparison to ELISA, Western blot allows detection of antibodies to virion proteins of distinct molecular weight. As an example, specific immunoreactivity to HTLV-I requires detection of antibodies to a core protein, gag p19 or p24, *and* an envelop protein, gp41 *(30)*. Along the same line, seroreactivity to HIV-1 is first tested by ELISA and confirmed by Western blot reactivity to a gag antigen, p24, and an envelope protein, gp41 or gp120. In response to antigenic stimulation, formation of IgM antibodies requires at least 2 wk, whereas high-titer IgG antibodies are generally detectable after 6–8 wk.

Polymerase-chain-reaction (PCR)-based detection of nucleic acids has been used for early diagnosis of viral infections, between viral exposure and production of detectable antibodies. Theoretically, a single viral RNA or DNA molecule may be sufficient for amplication by PCR. Viral DNA can be amplified with a set of sense and antisense primers. Viral RNA requires reverse transcription into complementary cDNA, prior to PCR. Reliable detection of viral sequences usually requires Southern blot hybridization with a probe internal to the location of oligonucleotide primers utilized for PCR. Gene amplification by PCR is currently the most sensitive diagnostic assay to detect any viral infection; nevertheless, it is not problem-free. Whereas DNA is fairly stable, RNA is prone to degradation by ubiquitous ribonucleases. Ironically, extreme sensitivity also represent an Achilles' heel of PCR-based methods. DNA contamination of clinical specimen can come form several sources: (1) another clinical specimen containing an abundant supply of target molecules for amplification by PCR, (2) contamination of reagents with PCR products, and (3) introduction of contaminants from skin, body fluids, or clothing of laboratory workers *(31)*. DNA products of PCR amplification should be handled separately from unprocessed clinical samples. Multiplex PCR allows detection of different viral nucleic acids in a single specimen. This technique is the diagnostic method of choice for testing of organs prior to transplantation. Quantitative PCR is useful for monitoring viral load in HIV-1 or HCV-infected patients in correlation with clinical course and responses to medications.

4. Viral Pathogenesis in Common/Idiopathic Autoimmune Diseases

Independent lines of evidence have implicated environmental and genetic factors in the development of autoimmune rheumatic diseases. A discordance of approximately 70% for SLE and RA in monozygotic twins argues for a significant role for exogenous agents. The possibility of a viral etiology was raised by findings of virionlike tubuloreticular structures in endothelial cells and lymphocytes as well as demonstration of elevated serum levels of type I interferon (IFN) in lupus patients *(32)*. Viruslike

particles were also noted in RA synovium *(33)*. Retroviruses were implicated by detection of retroviral p30 gag protein in renal glomeruli and serum reactivities towards p30 gag antigen in patients with SLE *(34)*. Many viral infections are accompanied by production of autoantibodies and viral proteins have profound effects on both antigen presentation and effector functions of the immune system. Dysregulation of programmed cell death has been documented in HIV-infected *(35)* and lupus patients as well *(36)*. Similar to SLE, anemia *(37)*, leukopenia *(38)*, thrombocytopenia *(39)*, polymyositis *(40)*, and vasculitis have been widely reported in patients with AIDS *(41)*. Direct virus isolation and transmission attempts from tissues of autoimmune patients have not been successful *(42)*. Nevertheless, it is possible that a (retro)virus, responsible for provoking an immune response cross-reactive with self-antigens, has been cleared from the host, so the absence of viral particles is not conclusive. An alternative retroviral etiology (i.e., activation of endogenous retroviral sequences [ERS]) was initially proposed by a study of the New Zealand mouse model of SLE *(43)*. Endogenous retroviral envelope glycoprotein, gp 70, was found in immune-complex deposits of autoimmune lupus-prone NZB/NZW mice *(43)*. Abnormal expression of an ERS was noted in the thymus of lupus-prone mouse strains *(44,45)*. More recently, expression and autoantigenicity of human ERS has been demonstrated in patients with SLE *(46–50)*.

Below, two lines of evidence for possible viral pathogenesis of autoimmunity will be reviewed. The first scenario involves molecular mimicry causing abnormal self-reactivity. Naturally, viral infections elicit potent antiviral immunity that may lead to crossreactivity against self-antigens. Analysis of molecular mimicries that is a delineation of autoantigenic epitopes of self-antigens may provide clues to the identity of viral antigens responsible for triggering the cross-reactive immune responses. Second, infection of genetically susceptible hosts by a potentially large number of commonly occurring viruses may lead to T- and B-cell dysfunction and autoimmunity. Immunoregulatory aberrations triggered by well-defined viral proteins at the level of antigen presentation, modulation of cytokine activities, and disruption of cell-death pathways, will be discussed.

4.1. Molecular Mimicry Between Viral Antigens and Self-Proteins

Molecular mimicry between self antigens and viral proteins has long been considered a trigger of autoimmunity *(51)*. Under normal conditions, the immune system of the host is able to develop a potent virus-specific immune response that rapidly eliminates the virus with only minimal tissue injury. Thus, only minimal amounts of self-antigens are released, which are insufficient to induce autoreactive B- and T-lymphocytes and autoimmune disease will not ensue. However, in the event that the host and the virus share antigenic determinants, virus infection may result in autoimmunity because virus-specific T-cells and antibodies are cross-reactive with self-antigens. This scenario does not preclude the possibility that the infecting virus is eliminated by the crossreactive immune response. Similarities between proteins of the major histocompatibility complex (MHC) and microbial antigens, especially viral antigens, may allow the host to regard an infectious agent as self and, thus, forego an immune response. Molecular mimicry (i.e., immunological crossreactivity between autoantigens and viral proteins) has been documented in human autoimmune disorders.

Table 2
Molecular Mimicry Between Viral Proteins and Autoantigens

Autoantigen	Prevalence[a]	Viral protein	Virus	Ref.
70k/U1 snRNP	30%	gag	MoMLV[b], HRES-1[c]	47,55
La	15%	gag	FSV	66
Sm B/B'	30%	gagp24	HIV-1	58
HRES-1	21–52%	gagp24	HTLV-I	46–49
C/U1 snRNP	30%	ICP4	HHV-1	64
Sm D	36%	EBNA-1	EBV	59
Sm B/B'	25–40%	EBNA-1	EBV	63
p542	10–50%	EBNA-1	EBV	60,61
ERV-3	32%	env	MoMLV	50

[a]Prevalence of antibodies in patients with SLE.
[b]MoMLV = Moloney murine leukemia virus.
[c]HRES-1 = human T-cell leukemia virus-related endogenous sequence 1.

The presence of the unique amino acid sequence QTDRED in the nitrogenase protein of Klebsiella and HLA B27 is thought to be a pathogenetic factor in seronegative spondylarthropathies *(52)*. Along the same line, the "shared epitope" QKRAA sequence from the third hypervariable region of HLA DRB1*0401, which has been found in numerous human pathogens, is associated with susceptibility to RA *(53)*.

A hallmark of the self-destructive autoimmune process in patients with SLE is the production of circulating antinuclear autoantibodies (ANAs). ANAs are important markers for diagnosis and classification and are possibly related causally or consequentially to the pathology of SLE. Targets of these antibodies include naked DNA and nuclear proteins involved in transcription and RNA processing. Autoantibodies to uridine-rich small nuclear ribonucleoproteins (UsnRNPs) frequently occur in patients with SLE and in overlap syndromes of SLE, scleroderma, and polymyositis (OLS) *(54)*. Sm (Smith) - type antibodies are directed to U1, U2, U5, and U4/U6 snRNPs, whereas RNP antibodies mainly react with different components of U1 snRNP *(54)*. The 70K protein of U1snRNP was the first lupus autoantigen shown to contain a region of homology and immunological crossreactivity with a conserved p30 gag protein of most mammalian-type C retroviruses (Table 2). Based on this observation, Query and Keene proposed that autoimmunity to U1RNP may be triggered by expression by an endogenous retroviral gag protein *(55)*. Anti-gag antibodies elicited by the ERS could crossreact with the 70K protein and, subsequently, recognition could expand to additional 70K epitopes.

The ERS capable of triggering antibodies crossreactive with the 70K protein may correspond to *HRES-1*, a human T-cell lymphotropic virus-related endogenous sequence *(47,56)*. In different laboratories, prevalence of HRES-1 antibodies may be as high as 52% *(47)* or as low as 21% *(49)* in patients with SLE. Fifty-nine percent (10/15) of scleroderma, 44% (8/18) of primary SJS, and 19% (3/16) of polymyositis/dermatomyositis patients also had HRES-1 antibodies. By contrast, 3.6% (4/111) of normal donors and none of 42 patients with AIDS or 50 asymptomatic HIV-infected patients had HRES-1 antibodies *(47)*. Thus, HRES-1 antibodies are detectable in a significant subset of autoimmune patients, whereas they are conspicuously absent in states of non-

specific polyclonal B-cell hyperactivity such as AIDS. The retroviral gag-related region of the 70K protein shares three consecutive highly charged amino acids, Arg-Arg-Glu (RRE), an additional Arg, and functionally similar Arg/Lys residues with HRES-1/p28, which represent crossreactive epitopes between the two proteins *(46,47,55)*. Interestingly, the RRE triplet is repeated three times in the 70K protein at residues 248–250, 418–420, and 477–479, respectively (GenEmbl accession number X04654). This suggests that recognition of the retroviral domain may lead to epitope spreading through binding to RRE triplets within the 70K protein. It is well known that highly charged polypeptides can elicit high-titer antibodies *(57)*. Therefore, the presence of charged amino acids in the mimicking epitopes may have important implications in triggering crossreactive antibodies of high affinity.

A mimicking epitope between another lupus autoantigen, Sm, and HIV-1 p24 gag was defined based on crossreactivity with monoclonal antibody 4B4 *(58)*. A proline-rich domain present in both the B/B' subunit of Sm and HIV p24 gag was suggested to be the core of crossreactive epitopes. Antibodies binding to HIV-1 p24 gag were found in 22/61 patients with SLE *(58)*. A region of considerable homology, comprised of 11 highly charged residues (GRGRGRGRGRG), was identified as a site of crossreactivity between the D component of Sm and the Epstein–Barr virus nuclear antigen 1 (EBNA-1) *(59)*. Mimicry between EBNA-1 and another self-protein, the 71 kD p542, has been revealed in patients with SLE and other autoimmune diseases *(60,61)*. The mimicking epitope, a 28-mer glycine-rich sequence, was selectively recognized by sera from autoimmune patients, whereas it was uncommonly targeted by sera from normal donors. Autoimmune sera recognized two additional epitopes of p542 in addition to its mimicking 28-mer. The concept that EBV can trigger IgG antibodies that crossreact with autoantigens is an attractive one. EBV is a ubiquitous human DNA virus which infects B cells and causes their polyclonal activation and thus polyclonal antibody production. Such polyclonal B-cell activation may be an early step in pathogenesis of SLE *(62)*. Interestingly, prevalence of EBV infection was reported to be as high as 99% in young SLE patients in comparison to a 70% prevalence in controls *(63)*. Therefore, EBV has the potential to trigger lupus by two mechanisms: polyclonal B-cell activation and molecular mimicry. The ICP4 protein of another ubiquitous human DNA virus, human herpesvirus type I (HHV-1), shows crossreactivity with the C component of U1 snRNP *(64)*.

Antibodies to p24 gag of HTLV-I were also noted in patients with SLE *(65)*, possibly secondary to crossreactivity with HRES-1 *(46)*. A region with limited sequence homology to the feline sarcoma virus (FSV) gag protein was noted in the La antigen *(66)*. Antibodies to the env protein of an ERS, ERV-3, were reported in patients with SLE with the highest prevalence in mothers of babies with complete heart block (CHB) *(50)*. A limited sequence similarity was revealed between the env of ERV-3 and MoMLV. Relationship between CHB-associated Ro/La and ERV-3 reactivities has not yet been directly addressed *(50)*.

Endogenous retroviral sequences, in addition to serving as crossreactive targets of antiviral immunity, may also have a direct role in regulating immune responses. ERS and other retrotransposable elements possess a relatively high mobility and thus represent a major factor in the shaping and reorganization of the eukaryotic genome *(67)*. The ERS HERV-K10 was found to have an integration site in the human complement C2 gene *(68)*. Variable repeats of this element may have a role in polymorphism and

differential expression of C2 loci. Integration of a 5.3-kb ETn retrotransposon in the FasR gene locus resulted in disruption of this apoptosis pathway in lupus prone MRL/lpr mice *(69,70)*. A synthetic heptadecapeptide corresponding to the transmembrane domain of the env protein conserved among many exogenous and endogenous retroviruses has been shown to have potent immunosuppressive properties *(71,72)*.

4.2. Viral Proteins Mimic Immunoregulatory Abnormalities of Autoimmune Patients

Tissue injury in rheumatic disease patients is often mediated by autoantibody-containing immune complexes. In turn, production of autoantibodies appear to be antigen-driven, polyclonal, and T-cell dependent *(73)*. A lack of tolerance to a variety of nuclear autoantigen is correlated with a profound dysfunction of both T- and B-cells. T-cell abnormalities include deficiencies of early activation events, proliferative responses to mitogens and antigens, T-helper, T-suppressor, and cell-mediated cytotoxic activities and decreased cell counts in the active stages of disease *(74,75)*. Functional abnormalities of T- and B-cells have been correlated with an altered cytokine production profile in patients with active SLE *(75)*. Secretion of T-helper type 1 (Th1) cytokines, interleukin-2 (IL-2), interferon-γ (IFNγ), and IL-12, necessary for maintenance of a classical T-cell-mediated immunity, is diminished *(75)*, whereas production of Th2 cytokines, in particular, IL-4, IL-5, IL-6 and IL-10, promoting B-cell function, is increased in patients with SLE *(76)*. This marked shift in cytokine production may be related to a fundamental biochemical defect manifested in deficiencies of protein kinase A activity, increased phosphatidylinositol turnover, and diminished protein kinase C activities in lupus T-cells *(74)*.

Changes in production of cytokines similar to those in patients with SLE have been described as a result of infection by HIV-1 *(77)*. Immune dysregulation in HIV-infected individuals observed during progression toward AIDS has been accounted for by a shift from a Th1-type to a Th2-type cytokine profile *(77)*. CD4 T-cell decline is mediated by an increased rate of apoptosis or programmed cell death (PCD) *(35)*. Interestingly, Th1-type cytokines protect against apoptosis, whereas Th2 cytokines increase PCD *(77)*. Accelerated apoptosis has also been described in SLE *(36)*. Moreover, apoptosis has been associated with a compartmentalized release of autoantigens in patients with SLE *(78)*. These observations raise the possibility that increased apoptosis and autoantibody production may be mediated by somewhat similar mechanisms both in AIDS and SLE.

Apoptosis or PCD represents a physiological mechanism for elimination of autoreactive lymphocytes during development *(79)*. Viral infections may have a role in dysregulation of apoptosis in autoimmune patients. Many viruses have evolved genes that can selectively inhibit or stimulate PCD. The suicide of an infected cell by internal activation of apoptosis or the killing of an infected cell by a cytotoxic T-lymphocyte or natural killer (NK) cell may be viewed as a defense mechanism of the host to prevent viral propagation. In the early stages of infection, viral inhibitors of apoptosis allow for more extensive production of progeny. At later stages, viral inducers of apoptosis facilitate spread of progeny to uninfected cells.

E1A of adenovirus *(80,81)* and E7 protein of human papilloma virus (HPV) activate p53-dependent apoptosis, which leads to elimination of virus-infected cells *(82)*. HIV

Table 3
Viral Proteins Stimulating Apoptosis

Protein	Virus	Pathway	Ref.
E1A	Adenovirus	Activates p53	80,81
E7	HPV	Activates p53	82
tat	HIV-1	Fas, oxidative stress	83,85
Protease	HIV-1	bcl-2 cleavage	86
tax	HTLV-I	bcl-2	100
ND[a]	Parvovirus B19	ND[a]	87
NS-1	Influenza	Fas, bcl-2	89

[a]ND = not determined.

may employ several mechanism to deplete CD4+ T-cells at the later stages of disease (Table 3). The tat protein induces oxidative stress *(83,84)* and enhances surface expression of the Fas ligand resulting in accelerated signaling through the Fas pathway *(83,85)*. In addition, cleavage of bcl-2 by HIV protease may expose the cell to a variety of apoptotic signals *(86)*. Parvovirus B19 depletes erythroid progenitor cells by apoptosis *(87)*, which raises the possibility of a similar mechanism in triggering arthritis in parvovirus-infected adults *(88)*. Cells infected by influenza virus undergo PCD that can be inhibited by bcl-2 and facilitated through the Fas pathway *(89)*. It is intriguing to consider the possibility that viruses causing common cold may stimulate antinuclear autoantibody production through periodic release of nucleosomes from apoptotic cells.

Inhibition of apoptosis by viral proteins help infected cells to evade inflammatory responses, such as killing by cytotoxic T-cells through the Fas and TNF pathways (Table 4). Moreover, blocking of cell-cycle-linked apoptotic mechanisms, mainly though interaction with the p53 tumor suppressor, can lead to increased viral replication and tumorigenesis. SV40 large T antigen binds directly to the p53 DNA binding region and blocks interactions with p53-specific promoter elements *(90)*. E6 of HPV promotes rapid degradation of p53 *(91)*. The pX protein of hepatitis B virus (HBV) inhibits binding of p53 to DNA by an unknown mechanism *(92)*. The *vpr* gene of HIV-1 causes cells to arrest in the G2 phase of the cell cycle when virus expression is highest *(93,94)*. *vpr* arrests cells in G2 by preventing activation/dephosphorylation of the p34cdc2/cyclin B complex that is required for entry into the M-phase. Therefore, *vpr*, by preventing p34cdc2 activation, may prevent apoptosis and, thus, increase viral replication in HIV-infected cells *(95)*. Viral homologs of bcl-2 can functionally substitute for bcl-2 in binding to the apoptosis-accelerating proteins, bax, bad, and bag *(96–98)*. Persistence of herpes simplex virus (HSV) in neurons has been linked to its apoptosis-inhibitory protein $\gamma_1$34.5 *(99)*. The p40/tax protein of HTLV-I appears to possess both apoptosis-inducing *(100)* and apoptosis-inhibiting capabilities *(101,102)*. A proposed role of p40/tax in blocking Fas-dependent cell death may be involved in autoimmune arthropathy *(103)* and Sjögren's syndrome documented in HTLV-I/tax-transgenic mice *(104)*. Upregulation of thioredoxin, a NADPH-dependent antioxidant *(101)*, and inhibition of Fas-dependent signaling have been implicated in the antiapoptotic effect of HTLV-I tax protein *(102)*. These two mechanisms are not

Table 4
Viral Proteins Inhibing Apoptosis

Protein	Virus	Pathway	Ref.
Large T	SV40	Inactivates p53	90
E1B 19K	Adenovirus	bcl-2 homolog	96
$\gamma_1$34.5	HSV	ND[a]	99
BHRF1	EBV	bcl-2 homolog	97
HMW5-HL	ASFV	bcl-2 homolog	98
pX	HBV	p53 antagonist	92
E6	HPV	p53 antagonist	91
p35, Iap	Baculovirus	Protease inhibitor	114
CrmaA	Cowpox	Protease inhibitor	106
vpr	HIV-1	Mitotic arrest	93,94
tax	HTLV-I	Fas, oxidative stress	101,102
23K E8-FLIP	EHV-2	Fas, vFLIP	107,108
ORF159L-FLIP	MCV	Fas, vFLIP	107,108
ORF71-FLIP	HVS	Fas, vFLIP	107,108
ORF189-FLIP	HHV-8	Fas, vFLIP	107,108

[a]ND = not determined.

mutually exclusive because Fas-induced cell death is accompanied by the formation of reactive oxygen intermediates (ROI) and is subject to regulation by enzymes of the pentose phosphate pathway, providing NADPH as a source of reducing equivalent for intracellular antioxidants *(105)*. The CrmA protein of the coxpoxvirus effectively blocks Fas- and TNF-induced cell death through inhibition of the family of IL1β-converting enzyme (ICE)-like cysteine proteases *(106)*. A new family of viral inhibitors, designated as vFLIPs (viral FLICE-inhibitory proteins), has recently been discovered *(107,108)*. vFLIPs are produced by several γ-herpesviruses, including the Kaposi-sarcoma-associated human herpesvirus 8 (HHV-8), the tumorigenic human molluscipoxvirus (MCV), herpesvirus saimiri (HVS), and equine herpesvirus 2 (EHV-2). vFLIPs block the early signaling events triggered through the death receptors Fas, TRAMP, TRAIL-R, and TNFR1. Thus, herpesviruses evolved a series of genes allowing selective blocking of the Fas- and TNFR-dependent signaling pathways.

5. Conclusions

The experimental evidence presented above reveals immunological crossreactivities between autoantigens and viruses. The concept that autoimmunity is triggered in genetically susceptible hosts by trivial environmental factors, possibly different from patient to patient, is consistent with the general epidemiology (i.e., a relatively sporadic occurrence) of the disease *(109)*. Moreover, proteins of commonly occurring viruses have profound effects on immune responses. Thus, molecular mimicry and immunomodulation by viral proteins may potentially account for both crossreactivity with autoantigens and abnormal T- and B-cell functions in autoimmune disorders. Therefore, continued research on viral pathogenesis is likely to provide new clues for understanding the causation of rheumatic diseases.

Acknowledgments

This work was supported in part by grant RO1 DK 49221 from the National Institutes of Health and grant RG 2466A1/3 from the National Multiple Sclerosis Society, the Arthritis Foundation, and the Central New York Community Foundation.

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