

Environmental Factors Involved in the Pathogenesis of Systemic Lupus Erythematosus

*Hiroshi Okamoto

Minami-Otsuka Institute of Technology, Minami-Otsuka Clinic, Tokyo, Japan

Abstract

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that predominantly affects women and is characterized by widespread immunologic abnormalities. The disease involves multiple organs including the skin, joints, and kidney, as well as the peripheral and central nervous systems. Significant progress has been made to elucidate the role of several loci and genes on the pathogenesis of SLE. In addition to the studies of genetic factors involved in the pathogenesis of SLE, progress has also been made on identifying environmental factors involved in the pathogenesis of this disorder. Therefore, overview of the environmental factors in the pathogenesis of SLE is quite informative for all researchers in the world. In this review, we discuss the historical and recent findings on the role of possible environmental factors such as sex hormones, viral infections (Epstein-Barr virus, Human Cytomegalovirus, Parvovirus B19, Retroviruses), ultraviolet radiation, cigarette smoking and alcohol consumption on SLE. In addition, I propose an interesting interaction between infectious agents, microRNAs and anti-dsDNA antibody production in the pathogenesis of SLE.

Keywords: Systemic lupus erythematosus, Sex hormones, Infectious agents, Ultraviolet

***Corresponding Author:** Hiroshi Okamoto, Minami-Otsuka Institute of Technology, Minami-Otsuka Clinic, Toshima-ku, Tokyo, Japan; Tel: +81-3-3943-7277; Fax: +81-3-3943-9018; E-mail: h-okamoto@dream.bbexcite.jp

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that predominantly affects women (90% of patients are women) and is characterized by a complex set of immunological abnormalities that result in dysfunction of several organs, including the skin, joints, kidney, serosal membranes, and central nervous system [1]. Recently, significant progress has been made towards elucidating the role of several loci and genes on the pathogenesis of SLE [2]. Extensive studies have made to elucidate the roles of T cells and cytokines/chemokines in the pathogenesis of SLE and these findings have impacted the development of therapeutic targets and biomarkers [3, 4]. In addition to the studies of genetic factors involved in the pathogenesis of SLE, progress has also been made in understanding the environmental factors involved in the pathogenesis of this disorder. Although overview of the environmental factors in the pathogenesis of SLE is quite important for all researchers in the world, little review articles have emphasized environmental factors thus far. To this end, we discuss the histological and recent findings on the role of environmental factors in the pathogenesis of SLE in this review.

Possible Environmental factors

Sex hormones

The predominance of females among the patients with SLE suggests the role of sex hormones in the immune system. It has been suggested that estrogens can enhance the immune response while androgens and progesterone suppress it. Many reports indicate that sex hormone receptors are expressed by various populations of immune cells. B cells have been shown to express both estrogen and androgen receptors while there is no evidence for progesterone receptor expression [5]. In T cells, only CD8⁺ T cells express estrogen receptors while CD4⁺ T cells do not express any of the steroid receptors [6]. Estrogen receptors were also found in monocytes and neutrophils [7, 8]. These data indicate that sex hormones can affect the function of the immune cells by binding to steroid receptors. Numerous lines of evidence suggest an important role for sex hormones in the adaptive immune system. Estrogen receptors α signaling has reported to regulate the development of Dendritic Cells (DCs), the antigen-presenting cells crucial for initiation of innate and adaptive immunity [9]. Sex-hormones have reported to affect T cell mediated immunity. The Th1/Th2 shift is one of the most important immunologic changes during gestation. It is due to the progressive increase of estrogens, which reach a peak level in the third trimester of pregnancy. At these high levels, estrogens suppress the Th1-mediated responses and stimulate Th2-mediated immunologic responses [10]. Progesterone has been shown to increase the cytokines produced by Th2 cells which predominate over those produced by Th1 cells, resulting in the maintenance of pregnancy. Th2 cells are dominant within the decidua in early pregnancy in humans. Progesterone has been shown to stimulate the secretion of Th2 and reduce the secretion of Th1 cytokines [11]. This observation might explain why Th1-mediated autoimmune diseases, such as rheumatoid arthritis, tend to improve and Th2-mediated diseases, such as SLE, tend to worsen during pregnancy.

Interestingly, a significant number of men suffering from SLE have higher estradiol levels and lower testosterone levels compared with healthy individuals [12].

Several studies indicate that changes in sex hormone levels caused by castration of animals influences the severity and/or the onset of different autoimmune diseases such as SLE. In (NZBxNZW) F1 mice (a murine model of SLE) the onset of disease is significantly delayed in males compared to females, while castration of males makes the onset of the disease similar to that in untreated female mice of the same strain. Similarly, ovariectomy of female (NZBxNZW) F1 mice significantly delays the onset of disease making it similar to that in untreated males of this strain [13]. These data suggest both protective roles for male hormones and disease accelerating properties of female hormones.

In vitro studies of human peripheral blood mononucleated cells (PBMCs) from SLE patients indicates that treatment of these cells with estrogen enhances total IgG production as well as anti-dsDNA autoantibody levels. This effect was partially interleukin (IL)-10 dependent and autoantibodies were not secreted by healthy control PBMCs treated in the same way. However, in a separate study, it was shown that estrogen induced total IgG and IgM production by PBMCs from healthy males and females while testosterone had the opposite effect [14]. These data suggest that sex hormones can directly affect the pathogenesis of autoimmune diseases by elevating the total level of immunoglobulins and enhancing autoantibody production. Furthermore, these observations indicate that female hormones can influence the onset of autoimmune disease. However, the studies of sex hormone levels in female patients with different autoimmune diseases did not reveal any significant differences to healthy controls. This indicates that, despite the important role of sex hormones in autoimmunity, there must be other factors such as infectious agents, cigarette smoking (describe later in this article) might explain the overall female bias of such diseases.

Infections

Epstein- Barr Virus (EBV)

EBV is a ubiquitous infectious agent, latently infecting approximately 95% of the world's population. Primary infection with EBV mostly occurs during childhood and causes a mild, usually asymptomatic infection. However, primary infection in adolescence causes infectious mononucleosis in 30–70% of cases, where upon to 20% of B-cells are infected with EBV. EBV is a DNA virus of the herpes family (human herpesvirus 4). It is comprised of a linear dsDNA genome enclosed by an icosahedral capsid, which is surrounded by the tegument and a host cell membrane-derived envelope embedded with glycoproteins. EBV has a fairly large genome coding for 87 proteins, and the functions of 72 of these are so far elucidated [15]. EBV is transmitted in saliva and initially infects epithelial cells in the oropharynx and nasopharynx. Subsequently, EBV enters the underlying tissues and infects B-cells [16]. After primary lytic infection, EBV persists in immortalized resting memory B-cells for the rest of the individual's life and can shift between an active lytic cycle and a latent state, from which it occasionally reactivates [17]. This ability of the virus to reactivate makes it a constant challenge to the host. In the latent state, the EBV genomic DNA will undergo circularization and replicate together with the host's chromosomal DNA, which results in a restricted expression of viral genes and conceals the virus from the host's immune system [18]. During the latent state, a maximum of nine genes are expressed including the EBV nuclear antigens (EBNA1, -2, -3A, -3B, and -3C), the Leader Protein (LP), and the Latent Membrane Proteins (LMP1, -2A, and -2B). EBNA1 is the only protein required for maintenance of the viral genome serving as a replication factor. When B-cells are latently infected for longer periods of time, EBV will only express EBNA1. EBNA2 is an important transcription factor during latency as it controls the expression of all other latent viral genes [19]. LMP1 and LMP2A rescue the infected B-cells from apoptosis, as they deliver the signal that normally comes from the CD40 signal transduction pathway initiated by CD4+ T-cell help (LMP1) and provide the

signal normally generated by antigen binding of the B-cell receptor (LMP2A) [20]. During lytic cycle of infection, EBV expresses numerous proteins involved in different viral activities. In the induction of lytic replication, two transcription factors, BZLF1 and BRLF1, activate early viral promoters required for generation of the initiation complex consisting of six viral proteins: the viral DNA polymerase (BALF5), the viral DNA polymerase accessory protein, early antigen diffuse (EA/D), a single-stranded DNA-binding protein (BALF2), the primase (BSLF1), the helicase (BBLF4) and the helicase/primase-associated protein (BBLF2/3). Several EBV proteins are involved in immune evasion mainly by inhibiting the interferon (IFN) pathways and T-cell immunity. An example is the viral IL-10 homologue, which, like human IL10, inhibits the synthesis of IFN γ and suppresses CD8+ cytotoxic T-cell responses as well as the upregulation of MHC I expression [21]. Furthermore, viral antiapoptotic proteins are expressed during the lytic cycle of infection including the early antigen restricted (EA/R) protein, which is a viral Bcl2 homologue that protects both infected B-cells and epithelial cells from apoptosis [22]. Many studies have linked EBV to the development of SLE. SLE patients have been shown to have a 10-40 fold increase in PBMC viralload compared to healthy controls [23]. The viral load was found to be associated with disease activity and to be independent of the intake of immunosuppressive medication. Furthermore, an elevated level of EBV DNA was found in serum from 42% of SLE patients compared to only 3% of healthy controls [24]. The findings on increased EBV load suggest active EBV lytic replication in SLE patients. As the viral load was associated with disease activity, it could be speculated that the reactivation of EBV is associated with development of SLE and flares.

Usually, little or no EBV mRNA is observed in normal immune competent carriers of EBV. However, several groups have demonstrated that SLE patients have abnormally high levels of several viral mRNAs (coding for BZLF1, gp350, viral IL10, LMP1, LMP2, and EBNA1). High expression of BZLF1 could imply reactivation of EBV, and increased gp350 could be

speculated to result in an amplified number of B-cells being infected with EBV. Furthermore, increased expression of viral IL-10 may give rise to enhanced immune evasion from the cell-mediated part of the immune system. In addition, an abnormal EBV latent state is also indicated by these results with improved survival of infected cells via enhanced expression of the LMP's [25].

Several lines of serologic evidence point to a connection between EBV infection and SLE. Antibodies to EBNA1, Viral Capsid Antigen (VCA), and EA in sera from SLE patients have been examined. Most studies find no difference between SLE patients and healthy controls in the prevalence of IgG and IgM antibodies to either EBNA1 or VCA [26]. However, studies on pediatric SLE patients and one adult study show that all SLE patients are seropositive for these antibodies compared to two-thirds of healthy controls [27]. Furthermore, elevated titers of IgG antibodies to EA/D, EA/R, and BALF2 have been observed in about half of SLE patients compared to only 8–17% of healthy controls [28]. Additionally, high levels of IgA antibodies to EA/D have been found in 58% of SLE patients and not in healthy controls. These results could not be explained by immunosuppressive medication, indicating that the antibodies are not produced upon reactivation of EBV due to an iatrogenically suppressed immune system. Presumably, these results reflect the attempt of the host to control reactivation or reinfection of EBV in epithelial cells [29].

EBV infection is mainly controlled by cell-mediated immunity. However, EBV-specific cytotoxic T-cell reactivity has been observed to be reduced in SLE patients resulting in poor control of the EBV infection. Less CD8⁺ cytotoxic T-cells were found to produce IFN- γ upon stimulation with EBV in the SLE patients compared to healthy controls, which must be a consequence of either defective or fewer EBV-specific cytotoxic T-cells [30]. Thus, SLE patients have an elevated viral load, increased EBV mRNA expression, elevated levels of EBV-directed antibodies, and decreased EBV-directed cell-mediated immunity compared to healthy controls, indicating poor control of EBV with frequent reactivation.

Human Cytomegalovirus (HCMV)

HCMV represents a human pathogenic herpes virus belonging to the subfamily of *Betaherpesvirinae*. As with all herpes viruses, HCMV has a large double-stranded DNA genome and possess a formidable coding capacity; giving rise to more than 750 translational products [31]. During coevolution over millions of years, HCMV adapted closely to the human host and today infects 40–99% of adult populations. After primary infection of diverse cell types (epithelial cells of the liver, lungs, kidney, salivary glands, smooth muscle cells, fibroblasts, etc.), HCMV remains latent in CD34⁺ myeloid progenitors, from which reactivation and recurrent replication can emerge. Even though a healthy immune system controls HCMV replication, the infection can neither be eliminated by immune functions nor by antiviral drugs, precluding a state of sterile immunity.

Studies performed in European countries found an association between HCMV and SLE disease [32]. However, several other studies did not observe a direct association between HCMV seroprevalence and SLE [33]. In one of these studies HCMV seropositivity correlated significantly with Raynaud's phenomenon [34]. Another study reported on significantly more frequent HCMV specific IgM in SLE patients than in controls, but no difference in HCMV IgG prevalence was observed [35]. This finding indicates that more frequent HCMV reactivation occurs in SLE patients, which may occur as a result of immunosuppressive treatment. In addition, other studies found higher frequencies of HCMV infection in SLE patients or higher HCMV IgG titers [36]. Moreover, in SLE patients with higher HCMV specific IgG titers more frequent autoantibodies could be detected [37]. An interesting study reported that a patient group positive for anti-HCMV IgM (and IgG) showed lower levels of autoantibodies against U1RNP/Sm and U1-70k in comparison to the HCMV IgM(-)/IgG(+) group, suggesting a role for HCMV reactivation in regulation of autoantibodies.

Altogether, these findings are compatible with the notion that genetic factors in combination with HCMV infection play an important role for SLE disease onset. Notably, antinuclear and

anti-dsDNA antibodies were found in patients suspected to have an onset of SLE as a consequence of HCMV infection [38]. The *UL83*-encoded pp65 matrix protein has been linked to autoantibodies in SLE patients. Elevated levels of anti-pp65 antibodies were found in SLE patients compared to controls and other patients with connective tissue disease. However, in SLE patients elevated levels of another tegument protein, pp150, were found raising the possibility that anti-CMV antibodies were generally induced in these patients. It was also reported that an *UL83*-encoding plasmid used for immunization of NZB/W F1 mice caused production of anti-dsDNA and antinuclear antibodies, leading to more severe signs of glomerulonephritis than control plasmid immunization [39].

One study with mice has shown that a HCMV gB expressing adenovirus induced antibodies against dsDNA and the U1-70kDa spliceosome (U1-70k) protein in immunized mice [40]. In humans, however, conflicting data are reported on U1-70k autoantibodies in healthy HCMV positive persons. Sera from healthy persons screened within a vaccination study were tested for the presence of U1-70k autoantibodies and an increase in frequency and quantity of HCMV infections were found [41]. The authors suggested that HCMV may play a role in inducing autoimmune disease in a subset of these individuals. In contrast, in a following study no indications for a higher frequency of SLE autoantibodies were found in sera from healthy volunteers vaccinated with the Towne strain [42].

Parvovirus B19

Human parvovirus B19 (B19V), a member of the *Parvoviridae* family, is a common human pathogen associated with a wide variety of diseases. Like all members of the *Parvoviridae* family, B19V is a small, nonenveloped virus (around 20 nm in diameter) [43]. The B19V core consists of 60 molecules of capsid proteins (viral protein [VP]). VP2 (58 kDa) is the major protein (95% of the capsid composition) and contains receptor- and coreceptor-binding domains as well as self-assembly domains that lead to the formation of highly stable particles. The main receptor of B19V is the P antigen and the $\alpha 5\beta 1$

integrin complex is clearly implicated as a coreceptor for the entry of B19V into permissive cells, such as erythroid progenitor cells [44, 45]. The Ku80 DNA-binding protein has also been implicated in B19V cell entry [46]. Interestingly, auto-antibodies against Ku antigen (anti-Ku) were originally described in patients with scleroderma-polymyositis overlap syndrome, several reports showed that anti-Ku antibodies are found also in patients with SLE.

Human parvovirus B19 infection is responsible for a wide range of human diseases, such as mild erythema infectiosum in immunocompetent children, fetal loss in primary infected pregnant women, and aplastic anemia or lethal cytopenias in immunocompromised adult patients. Since parvovirus B19 infection presents with multi- systemic symptoms resembling SLE both clinically and serologically, parvovirus B19 is the object of intense efforts to clarify whether it is also able to trigger autoimmune diseases. Similarities have been so striking that patients have been initially misdiagnosed with SLE, having fulfilled 3-5 of the criteria of the American College of Rheumatology. There are some reports suggesting the possibility that parvovirus B19 triggers autoimmunity in SLE.

Molecular mimicry between host and viral proteins seems to be one of the mechanisms involved in the induction of autoimmunity. By means of a random peptide library approach, Lunardi C et al. have identified a peptide that shares homology with parvovirus VP1 protein and with human cytokeratin. Moreover the VP peptide shares similarity with the transcription factor, GATA1 that plays an essential role in megakaryopoiesis and in erythropoiesis. These data support the role played by molecular mimicry in the induction of cross-reactive autoantibodies by parvovirus B19 infection [47].

Pavlovic's group designed a new fluorescence-based real-time method for monitoring anti-DNA antibody hydrolytic activity. By using an oligo-probe mimicking the thymidine pentamer part of the parvovirus B19 sequence, a region required which is a prerequisite for antibody binding to the substrate, the authors isolated and purified lupus anti-ssDNA antibody. It was found

that hydrolysis of the synthetic sequence occurs with different kinetics compared with commercial DNase1. This suggests that the antibody has intrinsic DNase activity that can cleave viral DNA. The hydrolysis of synthetic parvovirus B19 ssDNA sequence by lupus anti-ssDNA antibodies could be the molecular manifestation of an acquired antimicrobial attack of the host system, part of the anti-DNA antibody clearance strategy of the human body, or the direct antinuclear DNA cleavage during ssDNA replication within permissive host cells with resultant cell death and exposure to the immune system of the new array of antibodies [48].

Hsu-TC et al. reported that parvovirus B19 DNA was detected in 17 (24%) patients with SLE by PCR. This result was confirmed by Southern blotting. B19 DNA was not found in sera from patients with other autoimmune diseases, such as rheumatoid arthritis, Sjogren syndrome, or polymyositis. However, the presence of B19 DNA in patients with SLE may not be causative, it may rather reflect a superimposed B19 infection in patients with SLE due to lack of antibodies against B19. In this study, the prevalence of IgG and IgM anti-B19 antibodies in sera from SLE patients with B19 DNA was much lower than in patients without B19 DNA ($P < 0.05$). Furthermore, it has been demonstrated that the production of antibodies to the B19 capsid protein plays a major role in limiting parvovirus infection in human. In addition, there was no apparent association between the presence of B19 DNA and other clinical manifestations, such as skin rash, arthritis and proteinuria, in patients with SLE [49].

As these studies have not fully proved the role of this virus in the pathogenesis of SLE, further studies are required to elucidate how parvovirus B19 infection is involved in the pathogenesis of SLE, as well as how molecular mimicry, association of Ku autoantigen, and the triggering of anti-DNA antibodies are related.

Retroviruses

In recent years evidence has accumulated that supports a significant relationship between the so-called Human

Endogenous Retroviruses (HERV) and the development of different autoimmune diseases [50, 51]. HERVs are polynucleotide sequences representing the complete structure of the virus [52]. Their structure consists of 2 Long-Terminal Repeats (LTR). Between them there are the gene encoding the gag structural proteins, the genes encoding reverse transcriptase, protease, ribonuclease, and integrase (pol), the gene encoding the envelope proteins (env) and also the starter tRNA binding region (the primer binding site-PBS) as well as the so-called packaging signal (Ψ) – which is important for the function of the virus. HERVs were discovered in the 1980s. They represent about 8% of the human genome and are present in about 450 000 copies. They can be classified into 200 different groups and subgroups. HERVs were integrated into the germ-line cells of human ancestors. It is estimated that such insertions occurred 30 million years ago.

A specific type of HERVs can occur in the genome in a single copy, or can be repeated up to 1000 times. The greatest concentration of HERVs sequences is found in chromosomes Y, X, 4 and 20. HERVs is a member of the so-called retro-element family, these are nucleotide sequences that can move within the genome through a mechanism of “rewriting” their composition to RNA sequence and incorporation of its “DNA equivalent” in another location of the genome by the action of reverse transcriptase.

The majority of HERVs are not expressed, due to numerous inactivating mutations in the coding regions. These viruses are also frequently silenced by epigenetic mechanisms (DNA methylation). Some of the HERVs are active, however, and their expression is regulated by many different factors. Some types of HERVs are activated by X-rays and ultraviolet light. This occurs in patients with psoriasis. Pro-inflammatory cytokines, including IFN- α , TNF- α and IL-1 β and IL-6, and glucocorticoids can act as factors inducing activity of HERVs. Their expression is highest in the placenta tissue and endocrine glands (hypothalamus, male testes) [53].

Some of the HERVs are present in genes evolving rapidly with a high incidence of mutations, as occurs in genes involved in immune response processes. Surprisingly, the products of the expression of HERVs also have effects on physiological functioning and development of tissues. Li, F et al. reported that the *ERVWE1* locus on chromosome 7q harbors a member of the HERV-W family with an open reading frame in the *env* gene that encodes a protein denoted syncytin. This protein is highly expressed in the syncytiotrophoblast layer of the human placenta and appears to have been functionally adopted by the human host for fusion of trophoblast cells and thus contributing to the formation of the syncytiotrophoblast layer [54]. Thus, HERVs are helpful for the proper formation of the placenta and are involved in the suppression of rejection of fetal tissues. HERVs also interfere with exogenous viruses through interference with their receptors or the formation of antisense mRNA and are also related to the development of certain types of cancer [55].

Krzyształowska-Wawrzyniak et al. investigated the prevalence of HERV-K113 in patients with SLE, RA and in healthy subjects in a Polish population. Their data revealed statistically significant differences in the insertion frequencies of HERV-K113 between the groups of SLE and RA patients vs. healthy controls [56].

Baudino et al. examined murine Systemic Lupus Erythematosus (SLE) as a model of the pathogenesis of the human equivalent of this autoimmune disease [57]. Murine SLE is an autoimmune disorder characterized by B-cell hyperactivity leading to the production of various autoantibodies and subsequent development of glomerulonephritis. Among the principal targets of autoantibodies produced in murine SLE are nucleic acid-protein complexes (eg, chromatin) and the envelope glycoprotein gp70 of endogenous retroviruses. In NZB/W F1 mice, we have observed high expression of endogenous retroviruses ahead of the development of glomerulonephritis (unpublished observation). Recent studies revealed that the receptors TLR7 and TLR9 are involved in the activation of

autoreactive B-cells [58]. The regulation of autoimmune responses against endogenous retroviral gp70 by TLR7 suggests the involvement of endogenous retroviruses in this autoimmune response.

Perl and Fernandez found that the HRES-1 is a kind of HERV that encodes a 28-kD nuclear autoantigen and a 24-kD small GTP-ase, termed HRES-1/Rab4. HRES-1/p28 is a target of cross-reactive antiviral antibodies, whereas HRES-1/Rab4 regulates the surface expression of CD4 [59]. They concluded that HERVs can be considered as the molecular link between the human genome and environmental factors influencing the pathogenesis of SLE. HERV proteins may trigger lupus through structural and functional molecular mimicry, whereas the accumulation of HERV-derived polynucleotides stimulates interferon and anti-DNA antibody production.

One example of cross-talk between retroviral proteins and human proteins is the human immunodeficiency virus -1 Tat protein. HIV-1 encodes a regulatory protein, Tat, which is required for efficient transcription of viral genes. It enhances the processivity of RNA polymerase II by recruiting the positive elongation factor PTEF-b to the HIV-1 promoter through a Tat-TAR interaction. We have shown that the viral transactivator Tat inhibits the function of the class II transactivator CIITA, resulting in the suppression of expression of MHC class II genes in APC. On the other hand, we also have shown that the overexpression of CIITA can block the activity of Tat and HIV-1 replication. The reciprocal modulations between Tat and CIITA could explain the functional impairment of APC in HIV [60]. Similar interactions between retroviral proteins and human molecules might be essential for proper control of the immune response.

Infection, microRNAs and anti-DNA antibody production

MicroRNAs (MiRNAs) are ubiquitously involved in normal and diseased physiology including cancers, metabolic disorders, and infectious diseases [61]. In host-virus interactions, it has been suggested that RNAi serves as a critical component of the host

defense against viral infection in plants, invetebrate animals and mammals [62, 63]. MiRNAs represent a major class of small non-coding RNAs in the human genome. Humans encode for more than 1,600 characterized miRNAs. In the case of HIV-1 infection, many human miRNAs including miR-28, miR-29a, miR, miR-125b, miR-150 etc have been found to directly target HIV-1 sequences and to attenuate virus replication in cells, . Other cellular miRNAs have also been shown to indirectly target factors such as PCAF and cyclin T1 that are needed by HIV-1 to replicate [64]. Thus, these miRNAs can indirectly repress HIV-1 replication in cells. MiRNA-repression of the intracellular replication of mammalian viruses appears to be a common theme; indeed, an increasingly large number of published reports document the suppression of viruses such as Epstein Barr Virus (EBV), hepatitis B virus, human papilloma virus by various human miRNAs of [65-67].

The miRNA miR-155 has been extensively studied in relation to the regulation of immune responses. miR-155 is derived from the non-coding transcript of the proto-oncogene *bic* (B-cell integration cluster) and represents the only evolutionarily conserved sequence of this gene. miR-155, like other miRNAs, works by binding to the 3' untranslated region of mRNA via a protein complex called the RNA-induced silencing complex to effect either mRNA degradation or translational repression [68]. There are hundreds of potential target genes that miR-155 can regulate. Gracias *et al.* studied the role of miR-155 in the context of infection with influenza virus or *Listeria monocytogenes*. They found that miR-155 is highly expressed in effector and effector/memory CD8⁺ T cells but not in naive and central memory CD8⁺ T cells. Expression of miR-155 affected both optimal effector responses as well as development of a memory response [69]. The role of miR-155 has been studied in other infectious diseases such as HIV, hepatitis B virus and hepatitis C virus infection [70-72]. Interesting, an important connection between miR-155 and anti-dsDNA antibody production, aprerequisite for the development of SLE has been proposed [Figure 1].

DNA-containing ICs induce the activation and proliferation of autoreactive B cells through BCR and TLR9. BCR signaling activated the noncanonical NF- κ B pathway and enhanced the TLR-dependent canonical NF- κ B pathway, resulting in activation-induced cytidine deaminase (AID), which is critical for class-switch DNA recombination (CSR). After TLR9 stimulation of B cells, the relative concentrations of Ets-1 and Blimp-1 govern plasma cell formation. Early in the differentiation process, Ets-1 levels are high, and Blimp-1 levels are low, favoring the assembly of an Ets-1-Blimp-1 complex in which Blimp-1 is prevented from binding to its cognate DNA sequence. Ets-1 is capable of blocking DNA binding of Blimp-1, and might also affect the ability of Blimp-1 to repress target genes such as the B cell-specific promoter PIII of the major histocompatibility complex class II transactivator *CIITA*, the *pax-5* (BSAP) promoter, and the *c-myc* promoter. Later in differentiation Ets-1 levels fall, and Blimp-1 levels increase. Thus, Blimp-1 is relieved from the inhibitory effect of Ets-1, whereas activation of Blimp-1 target genes by Ets-1 is reduced. This allows effective repression of Blimp-1 target genes, thus driving plasmacyte differentiation. HMGB1, as a component of circulating DNA-containing ICs, could activate the TLR2/MyD88/miR-155 pathway and decrease Ets-1 expression, which in turn would contribute to plasmacytic differentiation by up-regulating the activity of Blimp-1 [73, 74]. Taken together, the human defense system against viral infections may have roles in the production of anti-DNA antibodies, and thereby resulting in the development of SLE.

Ultraviolet

Ultraviolet Radiation (UVR) is a well-known exacerbating factor for SLE, with photosensitivity comprising one of the American College of Rheumatology (ACR) diagnostic criteria for SLE. Cutaneous Lupus Erythematosus (CLE) has traditionally been sub-divided into acute, subacute, and chronic forms, according to the 1981 Gilliam and Sontheimer classification [75]. Further distinctions have been made regarding chronic forms, such as Discoid Lupus Erythematosus

(DLE), Lupus Tumidus (LET), lupus panniculitis/profundus, and chilblain lupus, among others.

Population incidence studies demonstrate that approximately 4.3 individuals per 100,000 are affected with CLE, and nearly two-thirds of patients meeting the diagnostic criteria for SLE will also have cutaneous manifestations of their disease. DLE remains the most common form of CLE, comprising over 75% of cases [76]. While acute cutaneous lupus erythematosus is most frequently associated with active systemic disease, up to 17% of those presenting with DLE may eventually develop SLE [77].

For those with CLE, photosensitivity is a well-documented symptom in which UV radiation is a major exacerbating factor in cutaneous lesion development. The association of UV and cutaneous lesions in lupus has been studied extensively to clarify the possible pathophysiology behind this phenomenon. UV promotes development of cutaneous lesions by augmenting lymphocytic recruitment and antibody-mediated cytotoxicity. Assessment of both ultraviolet-A (UVA) (320–400 nm) and ultraviolet-B (UVB) (290–320 nm) radiation suggest that each contributes via different mechanisms towards promoting cutaneous lesion development. UVB causes keratinocyte apoptosis by damaging DNA strand breaks and pyrimidine dimer formation. Though prior results have shown some conflicting reports, enhanced keratinocyte apoptosis in SLE patients has been observed in skin biopsy samples and *in vitro* cultures after UVB radiation, suggesting an increased susceptibility of keratinocytes to UVB damage and defective clearance over healthy skin [78]. UVB is also thought to play other pathologic roles via modulation of immunologic function and recruitment and attraction of inflammatory proteins, such as IL-1, TNF- α , intracellular adhesion molecule-1, and histocompatibility class II molecules [79, 80]. Other studies have suggested the enhanced translocation of lupus autoantigens to the cell surface of apoptotic keratinocyte blebs, aiding autoantibody exposure to these autoantigens, which are normally sequestered intracellularly [81].

Nitric Oxide (NO) is an important regulator of apoptosis and has an implication in the course of various autoimmune diseases. Interestingly, this molecule also has different effects on the various cell types within the skin, and it has been shown that NO can protect against UVA-induced apoptosis by increasing Bcl-2 expression and inhibiting UVA-induced overexpression of Bax protein in endothelial cells [82]. In addition, Weller et al. suggested an anti-apoptotic role for NO in keratinocytes after UVB irradiation [83]. Furthermore, UV exposure has also been shown to modulate local NO production through constitutive expression of neuronal nitric oxide synthase. Furthermore, several studies reported that another isotype of this family, the Inducible Nitric Oxide Synthase (iNOS), is expressed by epidermal keratinocytes after endogenous and/or exogenous stimuli. In 1998, it was demonstrated that iNOS is also expressed in human skin after UVA and UVB irradiation up to 48 h after exposure [84]. In striking contrast, an iNOS-specific signal appeared only 72 h after UV exposure and persisted in the evolving skin lesions of CLE patients for up to 25 days. These results suggest that the kinetics of iNOS induction and the time span of local iNOS expression might play an important role in the pathogenesis of this disease. It has further been reported that NO production is increased in patients with SLE possibly due to up-regulated iNOS expression in activated endothelial cells and keratinocytes [85].

Other pro-inflammatory effects are seen with UVA-induced damage. Pro-inflammatory cytokines such as IL-1 α/β and IL-6 are elevated following UVA exposure [86]. Similar to UVB, cyclobutane pyrimidine dimer formation is also seen [87]. UVA augments the binding of autoantibodies to keratinocyte surfaces, though to a lesser degree compared to UVB sensitization. Photosensitivity covers not only skin disease flares but also systemic symptoms such as fatigue and arthralgias [88].

Although the pathophysiological role of skin-infiltrating lymphocytes is clear, their recruitment and activation pathways in inflammatory skin diseases are still elusive. Recently, a

superfamily of small chemotactic proteins has been shown to regulate lymphocyte trafficking under inflammatory conditions, and it has been demonstrated that UV exposure induces the expression of T-cell attracting chemokines [89]. Furthermore, the CXCR3 ligands CXCL9, CXCL10 and CXCL11 have been identified as the most abundantly expressed genes in patients with CLE. Additionally, it has been reported that the CCR4 ligand TARC/CCL17 is strongly expressed in skin lesions and elevated in the serum of patients with CLE [90]. The functional relevance of lymphocytic CCR4 expression could be confirmed by TARC/CCL17-specific *in vitro* migration assays, suggesting that CCR4 and TARC/CCL17 play a role in the pathophysiology of this disease. We have reported that plasma TARC/CCL17 levels are elevated in human lupus patients and a murine model of lupus [91, 92].

Several photoprovocation studies have clearly demonstrated that the onset of true photosensitive reactions is often delayed, with one study reporting 78% of reactions occurring greater than one week after phototesting [93] and another showing the average onset occurring at eight days post-exposure. In fact, positive reactions have been observed up to three weeks after phototesting. Thus, because patients may not consider flares that occur days to weeks after extended sun exposure, we encourage providers to ask patients about flares that happen up to three weeks after being outside for an extended period. Kim A. et al. proposed that the definition for photosensitivity be the presence of a skin rash suggestive of cutaneous lupus and/or systemic symptoms such as fatigue or arthritis occurring up to 3 weeks after extensive sun exposure and lasting at least for several days or even weeks [94].

Other environmental factors

Cigarette smoking

Several epidemiologic studies have reported an increased risk of SLE among smokers. A meta-analysis of seven case-control studies and two cohort studies found that current smokers, but not former smokers, had a modest increased risk of SLE compared to nonsmokers (odds ratio (OR) 1.50, 95%

Confidence Interval (CI) 1.09–2.08) [95]. The case-control studies included a heterogeneous group with varying definitions of smoking status, questionnaire response rates, adjustment for potential confounders and timing of the study questionnaire in relation to the onset of SLE. Strikingly, in the case of the Hispanic cohort from New Mexico, the authors found a strong correlation between smoking and the incidence of SLE: an OR of 6.7 for current smokers and 3.7 for former smokers, whereas in other populations the ORs ranging from 0.9 to 2.3 for current smokers and 0.6 to 1.2 for former smokers [96]. Taken together, these results suggest that smoking status may confer an immediate risk for SLE, and that, with time after the cessation of smoking, the risk of SLE returns to that observed in those who have never smoked. Notably, two large prospective cohort studies, the Nurses' Health Study and the Black Women's Health Study, did not observe an association between cigarette smoking and the development of SLE [97, 98].

Active smoking may also affect disease severity in SLE. Ghaussy et al. studied the correlation of smoking status with disease activity (SLE Disease Activity Index score) and cumulative organ damage (SLE International Collaborating Clinics (SLICC)/American College of Rheumatology-Damage Index (ACR-DI) score). In a retrospective cohort analysis, investigators found that SLEDAI scores were significantly higher over a six-month period in current smokers compared to former and never smokers ($p < 0.001$). There was no significant difference in SLICC/ACR-DI scores [99].

The pathogenic role of smoking in autoimmune disease has extensively studied. In rheumatoid arthritis, we have shown that the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces inflammatory cytokines via its association with AhR, resulting in stimulation of the NF-kappaB and ERK signaling cascades [100].

Cigarette smoke contains numerous potentially toxic components, including tars, nicotine, carbon monoxide and polycyclic aromatic hydrocarbons. Exposure to such toxins, or their reactive metabolites, can directly damage endogenous proteins and DNA. In fact, cigarette smoke contains 10^{14} – 10^{16}

free radicals per puff—including reactive aldehydes, quinones and benzo (a)pyrene—which induce oxidative stress, implicated in the pathogenesis of SLE [101]. Cigarette smoke also induces epigenetic changes, some of which could modulate genes involved in pathways of inflammation and autoimmunity, perhaps triggering SLE [102]. Finally, exposure to cigarette smoke has harmful effects on both humoral and cell-mediated immunity. Cigarette smoke augments production of numerous pro-inflammatory cytokines such as TNF-alpha, IL-1, IL-6, IL-8 and granulocyte macrophage colony-stimulating factor while simultaneously decreasing the levels of anti-inflammatory cytokines such as IL-10 [103].

In a retrospective analysis of 140 ever-smokers and 270 nonsmokers with SLE, Freemer et al. found an association between current smoking and the presence of anti-double-stranded DNA (anti-dsDNA) antibodies. Compared to never smokers, current smokers were more likely to have dsDNA antibody seropositivity (OR 4.0, 95% CI 1.6–10.4). Former smokers, however, were not at increased risk for these antibodies compared to nonsmokers [104].

Mechanistic evidence implicating smoking in SLE pathogenesis is provided by three case-control studies from Japan, exploring potential gene-environment interactions. N-acetyltransferase 2 polymorphisms (responsible for hepatic detoxification of aromatic amines by acetylation), *CYP1A1* polymorphisms (a genotype promoting greater production of reactive oxygen species) and polymorphisms in the TNF receptor superfamily, member 1B (*TNFRSF1B*) were correlated with a significant increased risk of SLE [105-107].

Alcohol consumption

Several conflicting epidemiologic studies have investigated the relationship between alcohol consumption and risk of developing SLE. A case-control study by Hardy et al. was the first to report a statistically significant inverse association and dose-response relationship between alcohol consumption and SLE susceptibility, suggesting a possible protective effect [108]. Several other studies have suggested the possible protective

effect of alcohol in the pathogenesis of SLE. However, other investigators have reported conflicting results. In a meta-analysis of six case-control studies and one cohort study assessing the relationship between alcohol and SLE risk, Wang et al. found an overall significantly protective effect when all studies including patients with SLE treated for less than ten years were examined (OR 0.72, 95% CI 0.55–0.95). This report suggests a possible protective effect of moderate alcohol consumption on the development of SLE [109].

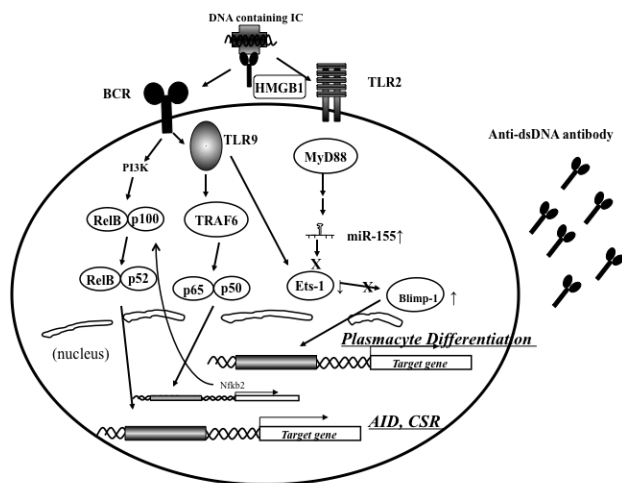
While ethanol contains many anti-inflammatory compounds, the exact mechanisms by which it might protect against SLE remain speculative [110]. Extensive evidence suggests that ethanol has dose-dependent immunomodulatory properties, wherein moderate use is associated with attenuated inflammation and heavy use is associated with increased inflammation [111]. Thus, moderate doses of alcohol might decrease susceptibility to inflammatory diseases by modulating synthesis and release of pro-inflammatory cytokines. For example, moderate alcohol consumption inhibits production of the pro-inflammatory cytokine IL-6, which is observed at high levels in patients with SLE and may be implicated in disease pathogenesis [112].

Conclusion

This review summarizes the involvement of environmental factors in the pathogenesis of SLE and suggests that not only genetic factors, but also environmental factors are important for the development of this disorder. Although various environmental factors may have direct roles in the pathogenesis of SLE, these direct effects alone could not be sufficient to account for the development of SLE. Indirect effects of environmental factors, such as cytokines/chemokines viral infection, molecular mimicry between host and pathogens also have roles in the pathogenesis of SLE. In addition, recent studies indicated the importance of host defense systems against infectious agents, such as miRNAs. Another mechanism of disease might be the squelching effect of nuclear factors by the proteins of infectious agents. For example, the HIV transactivator Tat and the transactivator of MHC class II genes,

CIITA, are mutually inhibitory by sequestering the common cofactor, cyclin T1 [60]. Further studies are required to draw a clearer picture of how environmental and genetic factors interact to result in the pathogenesis of SLE.

Figure: 1



Simplified schematic presentation for anti-dsDNA Ab production

DNA-containing ICs induce the activation and proliferation of autoreactive B cells through BCR and TLR9. BCR signaling activated the noncanonical NF- κ B pathway and enhanced the TLR-dependent canonical NF- κ B pathway, resulting in Activation-Induced cytidine Deaminase (AID), which is critical for class-switch DNA recombination (CSR). After TLR9 stimulation of B cells, the relative concentrations of Ets-1 and Blimp-1 govern plasma cell formation. Early in the differentiation process, Ets-1 levels are high, and Blimp-1 levels are low, favoring the assembly of an Ets-1-Blimp-1 complex in which Blimp-1 is prevented from binding to its cognate DNA sequence. Ets-1 is capable of blocking DNA binding of Blimp-1, it might also affect the ability of Blimp-1 to repress target genes such as the B cell-specific promoter PIII of the major histocompatibility complex class II transactivator *CIITA*, the *pax-5* (BSAP) promoter, and the *c-myc* promoter. Later in differentiation Ets-1 levels fall, and Blimp-1 levels increase. Thus, Blimp-1 is relieved from the inhibitory effect of Ets-1,

whereas activation of Blimp-1 target genes by Ets-1 is reduced. This allows effective repression of Blimp-1 target genes, thus driving plasmacyte differentiation. HMGB1, as a component of circulating DNA-containing ICs, could activate the TLR2/MyD88/miR-155 pathway and decrease Ets-1 expression, which contributes to plasmacytic differentiation by upregulating the activity of Blimp-1 [73, 74].

References

1. Okamoto H, Cervera R, Rodriguez-Reyna TS, Nishimura H, Yoshio T (2012) Systemic lupus erythematosus. *Autoimmune Dis*: 815753.
2. Moser KL, Kelly JA, Lessard CJ, Harley JB (2009) Recent insights into the genetic basis of systemic lupus erythematosus. *Genes Immun*. 10:373-379.
3. Tsokos GC, Kammer GM (2000) Molecular aberrations in human systemic lupus erythematosus. *Mol Med Today*. 6:418-424.
4. Okamoto H, Kobayashi A, Yamanaka H. (2010) Cytokines and chemokines in neuropsychiatric syndromes of systemic lupus erythematosus. *J Biomed Biotechnol*: 268436.
5. Benten WP, Stephan C, Wunderlich F (2002) B cells express intracellular but not surface receptors for testosterone and estradiol. *Steroids*; 67:647-654.
6. Stimson WH. (1988) Oestrogen and human T lymphocytes: presence of specific receptors in the T-suppressor/cytotoxic subset. *Scand J Immunol*. 28:345-350.
7. Suenaga R, Evans MJ, Mitamura K, Rider V, Abdou NI (1998) Peripheral blood T cells and monocytes and B cell lines derived from patients with lupus express estrogen receptor transcripts similar to those of normal cells. *J Rheumatol*; 25:1305-1312.
8. Molero L, García-Durán M, Diaz-Recasens J, Rico L, Casado S, et al. (2002) Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in

- neutrophils from women and men: regulation by estrogen. *Cardiovasc Res*. 56:43–51.
9. Kovats S. (2012) Estrogen receptors regulate an inflammatory pathway of dendritic cell differentiation: mechanisms and implications for immunity. *Horm Behav*. 62:254–262.
 10. Doria A, Iaccarino L, Sarzi-Puttini P, Ghirardello A, Zampieri S, et al. (2006) Estrogens in pregnancy and systemic lupus erythematosus; *Ann N Y Acad Sci*; 1069:247–256.
 11. Kumar P, Magon N. (2012) Hormones in pregnancy. *Niger Med J*; 53:179–183.
 12. Sequeira JF, Keser G, Greenstein B, Wheeler MJ, Duarte PC, et al. (1993) *Lupus*; 2:315–317.
 13. Gubbels Bupp MR, Jorgensen TN, Kotzin BL. (2008) Identification of candidate genes that influence sex hormone-dependent disease phenotypes in mouse lupus. *Genes Immun*; 9:47–56.
 14. Kanda N, Tsuchida T, Tamaki K. (1999) Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum*; 42:328–337.
 15. Farrell PJ. (2001) Epstein-Barr virus. The B95-8 strain map *Methods in Molecular Biology*; 174:3–12.
 16. Tsurumi T, Fujita M, Kudoh A. (2005) Latent and lytic Epstein-Barr virus replication strategies. *Reviews in Medical Virology*; 15:3–15.
 17. Thorley-Lawson DA. (2001) Epstein-Barr virus: exploiting the immune system. *Nat Rev Immunol*; 1:75–82.
 18. Tsurumi T, Fujita M, Kudoh A. (2005) Latent and lytic Epstein-Barr virus replication strategies. *Reviews in Medical Virology*; 15:3–15.
 19. Thorley-Lawson DA. (2001) Epstein-Barr virus: exploiting the immune system. *Nat Rev Immunol*; 1:75–82.
 20. Babcock GJ, Hochberg D, Thorley-Lawson DA. (2000) The expression pattern of Epstein-Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell. *Immunity*; 13:497–506.
 21. Kanegane H, Wakiguchi H, Kanegane C, Kurashige T, Tosato G. (1997) Viral interleukin-10 in chronic active Epstein-Barr virus infection. *J Infect Dis*; 176:254–257.
 22. Henderson S, Huen D, Rowe M, Dawson C, Johnson G, et al. (1993) Epstein-Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2, protects human B cells from programmed cell death. *Proc. Natl. Acad. Sci*; 90:8479–8483.
 23. Gross AJ, Hochberg D, Rand WM, Thorley-Lawson DA. (2005) EBV and systemic lupus erythematosus: a new perspective. *Journal of Immunology*; 174:6599–6607.
 24. Lu JJ-Y, Chen D-Y, Hsieh C-W, Lan J-L, Lin F-J, et al. (2007) Association of Epstein-Barr virus infection with systemic lupus erythematosus in Taiwan. *Lupus*; 16:168–175.
 25. Poole BD, Templeton AK, Guthridge JM, Brown EJ, Harley JB, et al. (2009) Aberrant Epstein-Barr viral infection in systemic lupus erythematosus. *Autoimmunity Reviews*. 8:337–342.
 26. Berkun Y, Zandman-Goddard G, Barzilai O, Boaz M, Sherer Y et al. (2009) Infectious antibodies in systemic lupus erythematosus patients. *Lupus*; 18:1129–1135.
 27. McClain MT, Poole BD, Bruner BF, Kaufman KM, Harley JB, et al. (2006) An altered immune response to Epstein-Barr nuclear antigen 1 in pediatric systemic lupus erythematosus. *Arthritis and Rheumatism*; 54:360–368.
 28. Huggins ML, Todd I, Powell RJ. (2005) Reactivation of Epstein-Barr virus in patients with systemic lupus erythematosus. *Rheumatology International*; 25:183–187.
 29. Draborg A, Jorgensen J, Muller H, Nielsen CT, Jacobsen S, et al. (2012) Epstein-Barr virus early antigen diffuse (EBV-EA/D)-directed immunoglobulin

- A antibodies in systemic lupus erythematosus patients. *Scand J Rheumatol*; 41:280–289.
30. Larsen M, Sauce D, Deback C, Arnaud L, Mathian A, et al. (2011) Exhausted cytotoxic control of epstein-barr virus in human lupus. *PLoS Pathogens*; 7; e1002328
 31. Stark TJ, Arnold JD, Spector DH, Yeo GW. (2012) High-resolution profiling and analysis of viral and host small RNAs during human cytomegalovirus infection. *Journal of Virology*; 86:226–235.
 32. Bendiksen S, van Ghelue M, Rekvig OP, Gutteberg T, Haga H-J, et al. (2000) A longitudinal study of human cytomegalovirus serology and viruria fails to detect active viral infection in 20 systemic lupus erythematosus patients. *Lupus*; 9:120–126.
 33. McClain MT, Poole BD, Bruner BF, Kaufman KM, Harley JB, et al. (2006) An altered immune response to Epstein-Barr nuclear antigen 1 in pediatric systemic lupus erythematosus. *Arthritis & Rheumatism*; 54:360–368.
 34. Stratta P, Canavese C, Ciccone G, Santi S, Quaglia M, et al. (1999) Correlation between cytomegalovirus infection and Raynaud's phenomenon in lupus nephritis. *Nephron*; 82:145–154.
 35. Su BY-J, Su C-Y, Yu S-F, Chen C-J. (2007) Incidental discovery of high systemic lupus erythematosus disease activity associated with cytomegalovirus viral activity. *Medical Microbiology and Immunology*; 196:165–170.
 36. Barzilai O, Sherer Y, Ram M, Izhaky D, Anaya JM, et al. (2007) Epstein-Barr virus and cytomegalovirus in autoimmune diseases: Are they truly notorious? A preliminary report. *Ann N Y Acad Sci*; 1108:567–577.
 37. Palafox Sánchez CA, Satoh M, Chan EK, Carcamo WC, Muñoz Valle JF, et al. (2009) Reduced IgG anti-small nuclear ribonucleoprotein autoantibody production in systemic lupus erythematosus patients with positive IgM anti-cytomegalovirus antibodies. *Arthritis Res Ther*; 11: R27.
 38. Ramos-Casals M, Cuadrado MJ, Alba P, Sanna G, Brito-Zerón P, et al. (2008) Acute viral infections in patients with systemic lupus erythematosus: Description of 23 cases and review of the literature. *Medicine*; 87:311–318.
 39. Chang M, Pan M-R, Chen D-Y, Lan J-L. (2006) Human cytomegalovirus pp65 lower matrix protein: A humoral immunogen for systemic lupus erythematosus patients and autoantibody accelerator for NZB/W F1 mice. *Clin Exp Immunol*; 143:167–179.
 40. Curtis HA, Singh T, Newkirk MM. (1999) Recombinant cytomegalovirus glycoprotein gB (UL55) induces an autoantibody response to the U1-70 kDa small nuclear ribonucleoprotein. *Eur J Immunol*; 29:3643–3653.
 41. Newkirk MM, van Venrooij WJ, Marshall GS. (2001) Autoimmune response to U1 small nuclear ribonucleoprotein (U1 snRNP) associated with cytomegalovirus infection. *Arthritis Research*; 3:253–258.
 42. Marshall BC, McPherson RA, Greidinger E, Hoffman R, Adler SP. (2002) Lack of autoantibody production associated with cytomegalovirus infection. *Arthritis Research*; 4, article R6
 43. Kaufmann, B, Simpson AA, Rossmann MG. (2004) The structure of human parvovirus B19. *Proc. Natl. Acad. Sci*; 101:11628–11633.
 44. Brown, K E, Anderson, SM, Young N.S. (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science*; 262:114–117.
 45. Weigel-Kelley, KA, Yoder, MC, Srivastava A. (2003) $\alpha 5 \beta 1$ integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of $\beta 1$ integrin for viral entry. *Blood*; 102:3927–3933.
 46. Munakata, Y., Saito-Ito, T, Kumura-Ishii, K, Huang, J, Kodera, T, et al. (2005) Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. *Blood*; 106:3449–3456.
 47. Lunardi C, Tinazzi E, Bason C, Dolcino M, Corrocher

- R, et al. (2008) Human parvovirus B19 infection and autoimmunity. *Autoimmun Rev*; 8:116-120.
48. Pavlovic M, Kats A, Cavallo M, Shoenfeld Y. (2010) Clinical and molecular evidence for association of SLE with parvovirus B19. *Lupus*; 19:783-792.
49. Hsu TC, Tsay GJ (2001) Human parvovirus B19 infection in patients with systemic lupus erythematosus.. *Rheumatology (Oxford)*; 40:152-157.
50. Balada E, Ordi-Ros J, Vilardell-Tarrés M. (2009) Molecular mechanisms mediated by human endogenous retroviruses (HERVs) in autoimmunity. *Rev Med Virol*; 19:273-286.
51. Balada E, Vilardell-Tarrés M, Ordi-Ros J. (2010) Implication of human endogenous retroviruses in the development of autoimmune diseases. *Int Rev Immunol*; 29:351-370.
52. Löwer R, Löwer J, Kurth R. (1996) The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. *Proc Natl Acad Sci*; 93:5177-5184.
53. Mi S, Lee X, Li X, Veldman GM, Finnerty H, et al. (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature*; 403:785-789.
54. Li F, Nellåker C, Yolken RH, Karlsson H. (2011) A systematic evaluation of expression of HERV-W elements; influence of genomic context, viral structure and orientation. *BMC Genomics*; 12:22.
55. Yi JM, Kim HM, Kim HS. (2004) Expression of the human endogenous retrovirus HERV-W family in various human tissues and cancer cells; *J Gen Virol*; 85:1203-1210.
56. Krzyształowska-Wawrzyniak M, Ostanek M, Clark J, Binczak-Kuleta A, Ostanek L, et al. (2011) The distribution of human endogenous retrovirus K-113 in health and autoimmune diseases in Poland. *Rheumatology (Oxford)*; 50:1310-1314.
57. Baudino L, Yoshinobu K, Morito N, Santiago-Raber ML, Izui S. (2010) Role of endogenous retroviruses in murine SLE. *Autoimmun Rev*; 10:27-34.
58. Santiago-Raber ML, Baudino L, Izui S. (2009) Emerging roles of TLR7 and TLR9 in murine SLE. *J Autoimmun*; 33:231-238.
59. Perl A, Fernandez D, Telarico T, Phillips PE. (2010) Endogenous retroviral pathogenesis in lupus. *Curr Opin Rheumatol*; 22:483-492.
60. Okamoto H, Asamitsu K, Nishimura H, Kamatani N, Okamoto T. (2000) Reciprocal modulation of transcriptional activities between HIV-1 Tat and MHC class II transactivator CIITA. *Biochem Biophys Res Commun*; 279:494-499.
61. Ebert MS, Sharp PA. (2012) Roles for microRNAs in conferring robustness to biological processes. *Cell*; 149:515-524.
62. Ding SW, Voinnet O. (2007) Antiviral immunity directed by small RNAs. *Cell*; 130:413-426.
63. Grassmann R, Jeang KT. (2008) The roles of microRNAs in mammalian virus infection. *Biochim Biophys Acta*; 1779:706-711.
64. Chiang K, Sung TL, Rice AP. (2012) Regulation of cyclin T1 and HIV-1 Replication by microRNAs in resting CD4+ T lymphocytes. *J Virol*; 86:3244-3252.
65. Riley KJ, Rabinowitz GS, Yario TA, Luna JM, Darnell RB, et al. (2013) EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. *EMBO J*; 31:2207-2221.
66. Liu WH, Yeh SH, Chen PJ. (2011) Role of microRNAs in hepatitis B virus replication and pathogenesis. *Biochim Biophys Acta*; 1809:678-685.
67. Zheng ZM, Wang X. (2011) Regulation of cellular miRNA expression by human papillomaviruses. *Biochim Biophys Acta*; 1809:668-677.
68. Bartel DP. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*; 136:215-233.
69. Gracias DT, Stelekati E, Hope JL, Boesteanu AC, Doering TA, et al. (2013) The microRNA miR-155 controls CD8+ T cell responses by regulating interferon signaling. *Nat Immunol*; 14:593-602.

70. Su C, Hou Z, Zhang C, Tian Z, Zhang J. (2011) Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. *Virology*; 8:354.
71. Bala S, Tilahun Y, Taha O, Alao H, Kodys K, et al. (2012) Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. *J Transl Med*; 10:151.
72. Seddiki N, Brezar V, Ruffin N, Lévy Y, Swaminathan S. (2014) Role of miR-155 in the regulation of lymphocyte immune function and disease. *Immunology*; 142:32-38.
73. John SA, Clements JL, Russell LM, Garrett-Sinha LA. (2008) Ets-1 regulates plasma cell differentiation by interfering with the activity of the transcription factor Blimp-1. *J Biol Chem*; 283:951-962.
74. Wen Z, Xu L, Chen X, Xu W, Yin Z, et al. (2013) Autoantibody induction by DNA-containing immune complexes requires HMGB1 with the TLR2/microRNA-155 pathway. *J Immunol*; 190:5411-5422.
75. Giliam JN, Sontheimer RD. (1981) Distinctive cutaneous subsets in the spectrum of lupus erythematosus. *J Am Acad Dermatol*. 4:471-475.
76. Durosaro O, Davis MD, Reed KB, Rohlinger AL. (2009) Incidence of cutaneous lupus erythematosus, 1965-2005: a population-based study. *Arch Dermatol*; 145:249-253.
77. Grönhagen CM, Fored CM, Granath F, Nyberg F. (2011) Cutaneous lupus erythematosus and the association with systemic lupus erythematosus: a population-based cohort of 1088 patients in Sweden. *Br J Dermatol*; 164:1335-1341.
78. Kuhn A, Herrmann M, Kleber S, Beckmann-Welle M, Fehsel K, et al. (2006) Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation. *Arthritis Rheum*; 54:939-950.
79. Furukawa F, Itoh T, Wakita H, Yagi H, Tokura Y, et al. (1999) Keratinocytes from patients with lupus erythematosus show enhanced cytotoxicity to ultraviolet radiation and to antibody-mediated cytotoxicity. *Clin Exp Immunol*; 118:164-170.
80. Köck A, Schwarz T, Kirnbauer R, Urbanski A, Perry P, et al. (1990) Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J Exp Med*; 172:1609-1614.
81. Golan TD, Elkon KB, Gharavi AE, Krueger JG. (1992) Enhanced membrane binding of autoantibodies to cultured keratinocytes of systemic lupus erythematosus patients after ultraviolet B/ultraviolet A irradiation. *J Clin Invest*; 90:1067-1076.
82. Suschek CV, Krischel V, Bruch-Gerharz D, Berendji D, Krutmann J, et al. (1999) Nitric oxide fully protects against UVA-induced apoptosis in tight correlation with Bcl-2 up-regulation. *J Biol Chem*; 274:6130-6137.
83. Weller R, Schwentker A, Billiar TR, Vodovotz Y. (2003) Autologous nitric oxide protects mouse and human keratinocytes from ultraviolet B radiation-induced apoptosis. *Am J Physiol Cell Physiol*; 284:C1140-C1148.
84. Kuhn A, Fehsel K, Lehmann P, Krutmann J, Ruzicka T, et al. (1998) Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. *J Invest Dermatol*; 111:149-153.
85. Belmont HM, Levartovsky D, Goel A, Amin A, Giorno R, et al. (1997) Increased nitric oxide production accompanied by the up-regulation of inducible nitric oxide synthase in vascular endothelium from patients with systemic lupus erythematosus. *Arthritis Rheum*; 40:1810-1816.
86. Wlaschek M, Heinen G, Poswig A, Schwarz A, Krieg T, et al. (1994) UVA-induced autocrine stimulation of fibroblast-derived collagenase/MMP-1 by interrelated

- loops of interleukin-1 and interleukin-6. *Photochem Photobiol*; 59:550–556.
87. Halliday GM, Byrne SN, Damian DL. (2011) Ultraviolet A radiation: its role in immunosuppression and carcinogenesis. *Semin Cutan Med Surg*; 30:214–221.
88. Zandman-Goddard G, Solomon M, Rosman Z, Peeva E, Shoenfeld Y. (2012) Environment and lupus-related diseases. *Lupus*; 21:241–250.
89. Meller S, Winterberg F, Gilliet M, Müller A, Lauceviciute I, et al. (2005) Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. *Arthritis Rheum*; 52:1504–1516.
90. Wenzel J, Henze S, Worenkamper E, Basner-Tschakarjan E, Sokolowska-Wojdylo M, et al. (2005) Role of the chemokine receptor CCR4 and its ligand thymus- and activation-regulated chemokine/CCL17 for lymphocyte recruitment in cutaneous lupus erythematosus. *J Invest Dermatol*; 124:1241–1248.
91. Okamoto H, Koizumi K, Yamanaka H, Saito T, Kamatani N. (2003) A role for TARC/CCL17, a CC chemokine, in systemic lupus erythematosus. *J Rheumatol*; 30:2369–2373.
92. Okamoto H, Nishimura H, Kamatani N. (2005) A role for TARC/CCL17, a CC chemokine, in New Zealand mice. *Rheumatology (Oxford)*; 44:819–820.
93. Sanders CJ, Van Weelden H, Kazzaz GA, Sigurdsson V, Toonstra J, et al. (2003) Photosensitivity in patients with lupus erythematosus: a clinical and photobiological study of 100 patients using a prolonged phototest protocol. *Br J Dermatol*; 149:131–137.
94. Kim A, Chong BF. (2013) Photosensitivity in cutaneous lupus erythematosus. *Photodermatol Photoimmunol Photomed*; 29:4–11.
95. Costenbader KH, Kim DJ, Peerzada J, Lockman S, Nobles-Knight D, et al. (2004) Cigarette smoking and the risk of systemic lupus erythematosus: A meta-analysis. *Arthritis Rheum*; 50: 849–857.
96. Ghaussy NO, Sibbitt WL Jr, Qualls CR. (2001) Cigarette smoking, alcohol consumption, and the risk of systemic lupus erythematosus: A case-control study. *J Rheumatol*; 28: 2449–2453.
97. Formica MK, Palmer JR, Rosenberg L, McAlindon TE. (2003) Smoking, alcohol consumption, and risk of systemic lupus erythematosus in the Black Women's Health Study. *J Rheumatol*; 30: 1222–1226.
98. Sánchez-Guerrero J, Karlson EW, Colditz GA, Hunter DJ, Speizer FE, et al. (1996) Hair dye use and the risk of developing systemic lupus erythematosus. *Arthritis Rheum*; 39: 657–662.
99. Ghaussy NO, Sibbitt W Jr, Bankhurst AD, Qualls CR. (2003) Cigarette smoking and disease activity in systemic lupus erythematosus. *J Rheumatol*; 30: 1215–1221.
100. Kobayashi S, Okamoto H, Iwamoto T, Toyama Y, Tomatsu T, et al. (2008) A role for the aryl hydrocarbon receptor and the dioxin TCDD in rheumatoid arthritis. *Rheumatology (Oxford)*; 47:1317–1322.
101. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. (2010) Markers of oxidative and nitrosative stress in systemic lupus erythematosus: Correlation with disease activity. *Arthritis Rheum*; 62: 2064–2072.
102. Costenbader KH, Gay S, Alarcón-Riquelme ME, Iaccarino L, Doria A. (2012) Genes, epigenetic regulation and environmental factors: Which is the most relevant in developing autoimmune diseases? *Autoimmun Rev*; 11: 604–609.
103. Arnsen Y, Shoenfeld Y, Amital H. (2010) Effects of tobacco smoke on immunity, inflammation and autoimmunity. *J Autoimmun*; 34: J258–J265.
104. Freemer MM, King TE Jr, Criswell LA. (2006) Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus. *Ann Rheum Dis*; 65: 581–584.

- 105.Kiyohara C, Washio M, Horiuchi T, Tada Y, Asami T,et al. (2009)Kyushu Sapporo SLE (KYSS) Study Group. Cigarette smoking, N-acetyltransferase 2 polymorphisms and systemic lupus erythematosus in a Japanese population. *Lupus*; 18: 630–638.
- 106.Kiyohara C, Washio M, Horiuchi T, Asami T, Ide S, et al (2012) Kyushu Sapporo SLE (KYSS) Study Group. Risk modification by CYP1A1 and GSTM1 polymorphisms in the association of cigarette smoking and systemic lupus erythematosus in a Japanese population. *Scand J Rheumatol*; 41: 103–109.
- 107.Kiyohara C, Washio M, Horiuchi T, Tada Y, Asami T, et al. (2009) Kyushu Sapporo SLE (KYSS) Study Group. Cigarette smoking, STAT4 and TNFRSF1B polymorphisms, and systemic lupus erythematosus in a Japanese population. *J Rheumatol*; 36: 2195–2203.
- 108.Hardy CJ, Palmer BP, Muir KR, Sutton AJ, Powell RJ. (1998) Smoking history, alcohol consumption, and systemic lupus erythematosus: A case-control study. *Ann Rheum Dis*; 57: 451–455.
- 109.Wang J, Pan HF, Ye DQ, Su H, Li XP.(2008) Moderate alcohol drinking might be protective for systemic lupus erythematosus: A systematic review and meta-analysis. *Clin Rheumatol*; 27: 1557–1563.
- 110.Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, et al. (2001) Effect of alcohol consumption on systemic markers of inflammation. *Lancet*; 357: 763–767.
- 111.Goral J, Karavitis J, Kovacs EJ. (2008) Exposure-dependent effects of ethanol on the innate immune system. *Alcohol*; 42: 237–247.
- 112.Ripley BJ, Goncalves B, Isenberg DA, Latchman DS, Rahman A. (2005) Raised levels of interleukin 6 in systemic lupus erythematosus correlate with anaemia. *Ann Rheum Dis*; 64: 849–853.