Sources of autoantigens in systemic lupus erythematosus

Kareem L. Graham and Paul J. Utz

Purpose of review

A hallmark of systemic lupus erythematosus is the production of autoantibodies that recognize nuclear antigens. However, the underlying events and mechanisms that lead to the selection of these molecules for the autoimmune response remain poorly understood. In this review, we will examine some of the proposed explanations for sources of systemic lupus erythematosus-specific autoantigens. We will focus on events related to apoptosis, viral infection, cytokine production, innate immune system components, and alternative splicing of pre-mRNA transcripts.

Recent findings

Definitive proof of a viral etiology for lupus remains elusive. However, recent observations have added to increasing evidence that viruses contribute to the bypass of tolerance in systemic lupus erythematosus. Also, events associated with apoptosis – most notably proteolytic autoantigen cleavage by caspases and granzyme B – have been implicated in the initiation of autoimmune responses for over a decade. Results obtained from animal models and human systems suggest complex functions for pro-apoptotic pathways in the regulation of immune responses. Inducible antigen expression and alternatively spliced transcripts may represent additional ways of generating autoantigenic material. Finally, toll-like receptor family members may play critical roles in the induction of antibody responses to nucleic acids in systemic lupus erythematosus.

Summary

Several factors may contribute to the generation of systemic lupus erythematosus-specific autoantigens. Determining the underlying causes of autoantibody production may provide important insight into the etiology and pathogenesis of this disease.

Keywords

autoantibodies, autoantigen, autoimmunity, systemic lupus erythematosus

Curr Opin Rheumatol 17:513–517. © 2005 Lippincott Williams & Wilkins.

Stanford University School of Medicine, Division of Immunology and Rheumatology, Stanford, California, USA

Correspondence to Paul J. Utz, MD, Stanford University School of Medicine, Division of Immunology and Rheumatology, CCSR Building, Room 2215-A, 269 Campus Drive, Stanford, CA 94305, USA
Tel: 650 724 5421; fax: 650 723-7509; e-mail: pjutz@stanford.edu

Current Opinion in Rheumatology 2005, 17:513–517

Abbreviations

ANA	antinuclear antibodies
CTL	cytotoxic T lymphocyte
EBNA-1	Epstein–Barr virus nuclear antigen-1
EBV	Epstein–Barr virus
IFNα	interferon-alpha
DNA-PK$_{CS}$	DNA-dependent protein kinase, catalytic subunit
PARP	poly (ADP-ribose) polymerase
SLE	systemic lupus erythematosus
TLR	toll-like receptor

1040-8711

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown etiology. Like most autoimmune diseases, the development of SLE is believed to be influenced by a combination of genetic, environmental, and hormonal factors [1].

SLE is characterized by the presence of antinuclear autoantibodies (ANAs), which are primarily directed against molecules that have roles in important cellular processes. A key issue in lupus is how these largely intracellular antigens become targets of the autoimmune response. The heterogeneous nature of SLE suggests that numerous factors may be involved in generating the autoantigens that are associated with this disease. Several findings point to a role for the pro-apoptotic protease granzyme B in breaking self-tolerance in SLE. It has been shown that granzyme B cleaves autoantigens that are targeted across the spectrum of human systemic autoimmune disease, producing unique autoantigen fragments that are not seen during caspase-mediated or other forms of cell death. These observations implicate granzyme B activity as a major contributor to the generation of novel, immunogenic epitopes. However, a number of SLE-associated autoantigens are inefficiently cleaved by granzyme B, indicating a possible role for other mechanisms. Although a viral etiology has been proposed for SLE and many other autoimmune diseases, formal proof of a viral origin for any autoimmune disorder remains difficult to establish. In this article, we will examine some of the prevailing hypotheses on the origins of autoantigens in SLE. We will focus on the role of proteolytic autoantigen cleavage by granzyme B, as well as two proposed mechanisms of virus-induced autoimmunity. We will also discuss the implications of inducible autoantigen expression, alternative splicing of autoantigen mRNA transcripts, and activation of toll-like receptor (TLR) family members (Table 1).

Apoptosis and autoimmunity

Apoptosis, or programmed cell death, is critical for immune system homeostasis and embryonic development,

Table 1. Possible sources of SLE-associated autoantigens

Event	Comments	References
Proteolytic autoantigen cleavage (granzyme B)	Attention has focused on the role of granzyme B in cryptic epitope generation, although other proteases (granzyme A, caspases, cathepsins, etc.) may participate in this process.	[20–25]
Environmental triggers (viruses)	Viruses may contribute to autoimmune responses in many ways, including (but not limited to) mimicry of host antigens, and direct modification of host proteins.	[30,31,36–38]
IFNα-inducible autoantigen expression	Other cytokines and conditions may also promote expression of 'novel' or 'untolerized' forms of self-antigens.	[46]
Alternative mRNA splicing	Autoantigen transcripts undergo noncanonical alternative splicing at high frequency.	[47]
Activation of TLR components	Chromatin has been implicated as an endogenous ligand for TLR9 on B cells, providing a possible explanation for autoantibodies directed against nucleic acids.	[49–51]

SLE, systemic lupus erythematosus; TLR, toll-like receptor.

and its morphologic and biochemical properties have been well-defined [2]. The link between apoptosis and autoimmunity has been widely studied, and several observations support a role for programmed cell death in inciting or propagating autoimmune disease. Ultraviolet irradiation – a common method of inducing apoptosis – results in the redistribution of lupus-associated autoantigens to the cell surface of cultured keratinocytes, where these antigens can then be recognized by human lupus sera, and presumably also by T and B cells [3,4]. In addition, immunization with apoptotic cells induces autoantibody production and glomerular IgG deposition in normal mice [5]. Numerous mechanisms have been proposed to explain how programmed cell death may contribute to the breakdown of immune tolerance. Excessive apoptosis in SLE patients may liberate DNA, histones, and other intracellular antigens that drive the autoimmune response [6]. Defects in the clearance of apoptotic debris may promote the release of antigens that are normally sequestered. These sequestered antigens may, in turn, trigger autoimmune responses [7]. The reduced phagocytic ability of macrophages derived from diseased lupus mice further supports a role for apoptotic cell removal in the progression of disease [8].

Notably, many lupus-associated autoantigens are posttranslationally modified during apoptosis. Examples include the La antigen which is dephosphorylated [9], and vimentin, which undergoes citrullination [10]. Thus, posttranslational modifications that occur during programmed cell death may allow these antigens to subvert normal mechanisms of peripheral tolerance, contributing to the immunogenicity of certain self-proteins in SLE (reviewed in [11]).

Proteolytic autoantigen cleavage by granzyme B

Proteolytic cleavage is an important component of a myriad of processes, including antigen processing and presentation [12] and regulation of cell signaling [13]. Caspases – cysteine proteases that cleave substrates immediately after aspartic acid residues – are the key effector molecules of apoptosis [14], and a small subset of lupus-associated autoantigens is cleaved by caspases during apoptosis. Examples include poly (ADP-ribose) polymerase (PARP) and the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{CS}), two autoantigens that play critical roles in DNA repair. It has been proposed that cleavage of these and other proteins may serve to abolish essential homeostatic activities and insure faithful execution of the apoptotic program [15]. However, proteolytic autoantigen cleavage may also serve as a general mechanism for the initiation of autoimmunity.

Engagement of the appropriate T lymphocyte or natural killer cell receptor triggers the release of cytotoxic granules. These granules contain perforin, a pore-forming protein, and serine proteases known as granzymes. Several granzymes have been identified, with granzymes A and B being the most abundant in mice and humans [16]. Granzyme B has been implicated in rapid induction of apoptosis, while granzyme A seems to play a more critical role in the later stages of programmed cell death [17–19]. Granzyme B cleaves directly after aspartic acid residues (similar to caspases), and is capable of activating caspase-dependent and caspase-independent pathways of cell death [16].

Casciola-Rosen *et al.* analyzed in detail the interaction of several autoantigens with granzyme B [20,21]. Although most of these autoantigens are also cleaved by one or more caspases, but incubation with granzyme B generates unique protein fragments that are not seen during caspase-mediated forms of cell death [21]. Importantly, 'non-autoantigens' do not appear to be substrates for granzyme B. Also, the tissue where an autoantigen is expressed may influence its susceptibility to proteolysis [22].

In support of a role for granzyme B in triggering autoimmune responses, it has been reported that human anti-centromere protein B autoantibodies selectively bind granzyme B-generated autoantigen fragments [23]. In addition, studies performed by Blanco *et al.* suggest that $CD8^+$ cytotoxic T lymphocyte (CTL) effector status correlates with SLE disease activity. $CD8^+$ CTLs isolated from SLE patients generated unique granzyme B-dependent autoantigen

fragments upon incubation with K562 human erythroleukemia cells [24]. These results suggest a pathogenic role for granzyme B in SLE. However, granzyme B is not required for the initiation of autoantibody responses after immunization with the mineral oil pristane. In the pristane-induced model of SLE, granzyme B-deficient mice produce autoantibodies to the U1-70 kDa antigen, a well-characterized unique substrate for granzyme B. Pristane-primed mice also produce antibodies to the nuclear factor 90 antigen, a novel substrate for granzyme B [25]. Differences between mouse and human enzymes and autoantigens may reconcile these seemingly disparate findings. Also, the pristane-induced pathway of lupus may differ from human disease, as the mechanism whereby pristane triggers lupus-like autoimmunity remains unknown. It is also unclear whether this mineral oil induces activation of the perforin/granzyme pathway. However, immunization with squalene, a precursor of cholesterol, has been shown to induce lupus autoantibodies in mice. These observations suggest that the ability to stimulate autoimmunity is shared by chemically diverse hydrocarbon oils, and indicate that the pristane model is of some physiologic relevance [26]. In addition, the observation that $CD4^+$ regulatory T cells can use the perforin pathway to kill autologous target cells suggests a regulatory role for cytotoxic granule components in humans [27••]. Together, the data indicate that perforin and granzyme B have complex roles in regulating immune responses.

Molecular mimicry in systemic lupus erythematosus

Many diseases that are characterized by autoimmune phenomena may actually be infectious in nature. In support of this view, viral infections are associated with a variety of autoimmune conditions, including multiple sclerosis and type I diabetes [28]. However, the mechanisms responsible for virus-induced autoimmunity remain poorly understood.

Molecular mimicry – defined as cross-reactivity between microbial and self-determinants recognized by the adaptive immune system – is perhaps the most popular explanation for the clinical association between microbial infection and autoimmune disease [28]. Epstein–Barr (EBV) virus infection has long been linked with SLE [29]; however, the significance of this association has not been entirely clear. Previously, James and Harley *et al*. have noted similarity between a region of the EBV nuclear antigen-1 (EBNA-1) and an epitope of the Sm-BB′ autoantigen. Immunization of rabbits with the Sm-BB-derived PPPGM RPP and PPPGIRGP octapeptides, which resemble the PPPGRRP epitope of EBNA-1, induced autoantibodies to other regions of the Sm-BB′ protein, as well as epitope spreading to other spliceosomal components [30]. Recently, a potential role for EBV-induced molecular mimicry in the initiation of SLE has been reexamined. In an elegant study, McClain *et al*. analyzed serum samples collected from lupus patients prior to their diagnosis with clinical disease. These authors determined that antibodies directed against the initial epitope of the human Ro-60-kDa (Ro-60) autoantigen directly cross-react with a region of EBNA-1. Interestingly, the initial Ro-60 epitope shares no primary sequence homology with the EBNA-1 linear epitope. Rabbits immunized with the first epitope of Ro-60 or the cross-reactive EBNA-1 epitope developed autoantibodies directed against multiple epitopes of Ro and spliceosomal autoantigens, and eventually developed clinical symptoms of lupus [31••].

Taken together, these observations provide strong support for the hypothesis that anti-Ro-60 and anti-Sm-BB′ autoantibodies in human lupus arise through molecular mimicry. However, this hypothesis would only account for autoantibodies seen in a subset of SLE patients [32•]. There may be other cross-reactive regions in EBNA-1 or different EBV proteins that are involved in triggering SLE. Furthermore, EBV is extremely prevalent, with more than 90% of the world's population presumed to serve as carriers [33]. Therefore, other factors – genetic, hormonal, environmental, or stochastic – must play a role in EBV-induced autoimmune responses. Other viruses may also promote SLE.

Viral proteases

Other mechanisms for virus-induced autoimmunity have been proposed, including presentation of virus/self-protein complexes to autoreactive lymphocytes [34] and bystander activation [35]. In addition, the phenomenon of autoantigen cleavage by viral proteases may represent another relevant mechanism for virally induced autoimmunity.

An important event in the life cycle of many viruses is the interaction of virus-encoded proteases with host cell proteins. This can result in site-specific cleavage of molecules that have pivotal roles in host cell metabolism, thereby promoting viral replication and viral release from infected cells. In addition to inhibiting host cell transcription and translation, proteolytic cleavage of host proteins by viral proteases may have another consequence: the generation of novel self-epitopes that can trigger autoimmune responses. Importantly, such a scenario would not exclude bystander activation, molecular mimicry, or other potentially valid mechanisms.

To date, a limited number of autoantigens have been identified as substrates for viral proteases. These include histone H3, a substrate for a foot-and-mouth disease virus protease [36], as well as the La antigen, which is cleaved by the poliovirus 3C protease [37]. Recently, we have shown that DNA-PK_{CS} is cleaved by a picornavirus 2A protease [38], indicating that viral proteases may contribute to the generation of novel autoantigenic epitopes. More-comprehensive screens may identify additional substrates for viral proteases. However, the immunogenicity of these protease-generated cleavage fragments must be formally

determined. As a corollary to this, a number of important substrates for viral proteases – such as poly (A) binding protein and TATA-binding protein – have not been identified as autoantigens in human disease. Therefore, the precise contribution of autoantigen cleavage by viral proteases to the bypass of self-tolerance remains to be established. The current data suggest that virus-induced autoimmune responses are likely to result from interplay between several factors. These may include molecular mimicry, novel epitope generation by viral proteases, and the proinflammatory conditions that are associated with viral infection. Similarly, granzymes are expressed by cells that have important roles in controlling viruses and tumors. Autoimmunity arising as a result of granzyme B activity may stem from a legitimate immune response to a microbe, malignancy, or some other insult.

Inducible autoantigens and alternative splicing

Microarray analysis of blood mononuclear cells derived from SLE patients has identified an interferon biosignature. This biosignature is characterized by the presence of elevated transcripts for interferon-inducible genes [39,40]. In particular, there is considerable literature supporting a major pathogenic role for type I interferons such as interferon-alpha (IFNα) (reviewed in [41•]). Notably, IFNα has been shown to induce dendritic cell differentiation [42], and plasmacytoid dendritic cell (pDC) production of IFNα has been implicated in the activation of plasma cells [43].

Observations from several other groups have also focused attention on IFNα as a central regulator of SLE. However, the role of type I interferons in murine lupus models is somewhat controversial. NZB mice lacking the α-chain of the IFNα/β receptor develop less severe clinical disease than their wild-type counterparts [44]. Conversely, deficiency in this same subunit exacerbates disease in the MRL/lpr model of lupus nephritis [45]. It has been shown that some of the autoantigens targeted by autoantibodies in a transgenic murine lupus model are IFNα-inducible [46]. These results suggest that some self-antigens may be selected for autoimmune responses when their expression is induced by specific cytokines or conditions in the periphery.

Recent studies have also implicated a role for transcript splicing in the initiation of SLE and other autoimmune diseases [47••]. Ng *et al*. noted that autoantigen transcripts undergo alternative splicing at greater frequency than transcripts of non-autoantigens. These alternatively spliced isoforms may encode novel ('untolerized') epitopes that are not expressed in central lymphoid organs. The events that regulate expression of these untolerized isoforms remain to be determined. It is also unclear whether the proteins encoded by alternatively spliced transcripts are preferentially recognized by autoimmune sera or autoreactive T lymphocytes. However, these observations suggest yet another intriguing potential source of autoantigens in SLE.

Immunongenecity of nucleic acids

Members of the mammalian TLR gene family are involved in the recognition of various microbial components and products. Most TLRs are located on the plasma membrane; however, activation of TLR9 takes place in intracellular compartments [48]. TLR9 is engaged by hypomethylated CpG motifs, commonly found in bacterial DNA. However, co-engagement of the B cell receptor (BCR) and TLR9 by mammalian chromatin-containing immune complexes may play a pivotal role in the activation of autoreactive B cells [49]. Recently, chromatin (not bound by antibody) has been implicated as an endogenous ligand for TLR9. In this model, chromatin (released from apoptotic cells, for example) sequentially engages the BCR and TLR9. This leads to activation of chromatin-reactive B cells and eventual formation of chromatin-containing immune complexes. These immune complexes may further activate B cells, dendritic cells, and pDCs, thus providing a link between the innate and adaptive immune systems in the production of anti-DNA antibodies [50]. Mechanisms that may be involved in rendering mammalian chromatin immunogenic have been described [51]. Future studies may implicate other TLRs (e.g. TLRs 3, 7, and 8) in the development of autoantibodies to RNA and RNA-associated complexes.

Conclusion

Studies aimed at uncovering mechanisms involved in SLE pathogenesis are complicated by the incredibly diverse nature of the disease. It appears that no single gene is necessary or sufficient for complete disease expression, and the role of specific environmental contributions is difficult to pinpoint. There is a tremendous need to clarify the relative importance of the factors involved in the initiation of SLE. Doing so may facilitate the development of rational preventive and therapeutic approaches in the clinical setting.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Tsao BP. The genetics of human systemic lupus erythematosus. Trends Immunol 2003; 24:595–602.

2 Albert ML. Death-defying immunity: do apoptotic cells influence antigen processing and presentation? Nat Rev Immunol 2004; 4:223–231.

3 LeFeber WP, Norris DA, Ryan SR, *et al*. Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured keratinocytes. J Clin Invest 1984; 74:1545–1551.

4 Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface blebs on cultured keratinocytes. J Exp Med 1994; 179:1317–1330.

5 Mevorach D, Zhou J, Song X, *et al*. Systemic exposure to irradiated apoptotic cells induces autoantibody production. J Exp Med 1998; 188:387–392.

6 Rosen A, Casciola-Rosen L. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. Cell Death Differ 1999; 6:6–12.

7 Carroll MC. A protective role for innate immunity in systemic lupus erythematosus. Nat Rev Immunol 2004; 4:825–831.

8 Licht R, Dieker JW, Jacobs CW, *et al*. Decreased phagocytosis of apoptotic cells in diseased SLE mice. J Autoimm 2004; 22:139–145.

9 Rutjes SA, Utz PJ, Broekhuis C, *et al*. The La (SS-B) autoantigen, a key protein in RNA biogenesis, is dephosphorylated and cleaved early during apoptosis. Cell Death Differ 1999; 6:976–986.

10 Asaga H, Yamada M, Senshu T. Selective deimination of vimentin in calcium ionophore-induced apoptosis of mouse peritoneal macrophages. Biochem Biophys Res Commun 1998; 243:641–646.

11 Utz PJ, Gensler TJ, Anderson P. Death, autoantigen modifications, and tolerance. Arthritis Res 2000; 2:101–114.

12 Villadangos JA, Ploegh HL. Proteolysis in MHC class II antigen presentation: who's in charge? Immunity 2000; 12:233–239.

13 Weihofen A, Martoglio B. Intramembrane-cleaving proteases: controlled liberation of proteins and bioactive peptides. Trends Cell Biol 2003; 13:71–78.

14 Garrido C, Kroemer G. Life's smile, death's grin: vital functions of apoptosis-executing proteins. Curr Opin Cell Biol 2004; 16:639–646.

15 Casciola-Rosen L, Nicholson D, Chong T, *et al*. Apopain/CPP32 cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. J Exp Med 1996; 183:1957–1964.

16 Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. Nat Rev Immunol 2003; 3:361–370.

17 Heusel JW, Wesselschmidt RL, Shresta S, *et al*. Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells. Cell 1994; 76:977–987.

18 Simon MM, Hausmann M, Tran T, *et al*. In vitro- and ex vivo-derived cytolytic leukocytes from granzyme A x B double knockout mice are defective in granule-mediated apoptosis but not lysis of target cells. J Exp Med 1997; 186:1781–1786.

19 Shresta S, Graubert TA, Thomas DA, *et al*. Granzyme A initiates an alternative pathway for granule-mediated apoptosis. Immunity 1999; 10:595–605.

20 Andrade F, Roy S, Nicholson D, *et al*. Granzyme B directly and efficiently cleaves several downstream caspase substrates: implications for CTL-induced apoptosis. Immunity 1998; 8:451–460.

21 Casciola-Rosen L, Andrade F, Ulanet D, *et al*. Cleavage by granzyme B is strongly predictive of autoantigen status. Implications for initiation of autoimmunity. J Exp Med 1999; 190:815–826.

22 Ulanet DB, Flavahan NA, Casciola-Rosen L, *et al*. Selective cleavage of nucleolar autoantigen B23 by granzyme B in differentiated vascular smooth muscle cells: insights into the association of specific autoantibodies with distinct disease phenotypes. Arthritis Rheum 2004; 50:233–241.

23 Schachna L, Wigley FM, Morris S, *et al*. Recognition of Granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. Arthritis Rheum 2002; 46:1873–1884.

24 Blanco P, Pitard V, Viallard JF, *et al*. Increase in activated CD8+ T lymphocytes expressing perforin and granzyme B correlates with disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 2005; 52:201–211.

25 Graham KL, Thibault DL, Steinman J, *et al*. Granzyme B is dispensable for immunological tolerance to self in a murine model of systemic lupus erythematosus. Arthritis Rheum 2005; 52:1684–1693.

26 Satoh M, Kuroda Y, Yoshida H, *et al*. Induction of lupus autoantibodies by adjuvants. J Autoimm 2003; 21:1–9.

27 •• Grossman WJ, Verbsky JW, Barchet W, *et al*. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. Immunity 2004; 21:589–601.

This study provides the first evidence that human T regulatory cells use the perforin/granzyme pathway to suppress autoimmune responses.

28 Christen U, von Herrath MG. Induction, acceleration or prevention of autoimmunity by molecular mimicry. Mol Immunol 2004; 40:1113–1120.

29 James J, Kaufman K, Farris A, *et al*. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. J Clin Invest 1997; 100:3019–3026.

30 James JA, Gross T, Scofield RH, *et al*. Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. J Exp Med 1995; 181:453–461.

31 •• McClain MT, Heinlen LD, Dennis GJ, *et al*. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. Nat Med 2005; 11:85–89.

This study provides perhaps the best evidence to date of a role for EBV-induced molecular mimicry in SLE.

32 • Sherer Y, Gorstein A, Fritzler MJ, *et al*. Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum 2004; 34:501–537.

This is a comprehensive review of the autoantibodies associated with SLE, along with their clinical associations, correlations with disease activity, and other relevant information.

33 Macsween KF, Crawford DH. Epstein-Barr virus - recent advances. Lancet Infect Dis 2003; 3:131–140.

34 Dong X, Hamilton K, Satoh M, *et al*. Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. J Exp Med 1994; 179:1243–1252.

35 Christen U, von Herrath MG. Initiation of autoimmunity. Curr Opin Immunol 2004; 16:759–767.

36 Falk M, Grigera P, Bergmann I, *et al*. Foot-and-mouth disease virus protease 3C induces specific proteolytic cleavage of host cell histone H3. J Virol 1990; 64:748–756.

37 Shiroki K, Isoyama T, Kuge S, *et al*. Intracellular redistribution of truncated La protein produced by poliovirus 3Cpro-mediated cleavage. J Virol 1999; 73:2193–2200.

38 Graham KL, Gustin KE, Rivera C, *et al*. Proteolytic cleavage of the catalytic subunit of DNA-dependent protein kinase during poliovirus infection. J Virol 2004; 78:6313–6321.

39 Baechler E, Batliwalla F, Karypis G, *et al*. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci USA 2003; 100:2610–2615.

40 Bennett L, Palucka A, Arce E, *et al*. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 2003; 197:711–723.

41 • Banchereau J, Pascual V, Palucka AK. Autoimmunity through cytokine-induced dendritic cell activation. Immunity 2004; 20:539–550.

The authors present their perspective on the role of cytokines in regulating the balance between protective immunity and tolerance.

42 Blanco P, Palucka AK, Gill M, *et al*. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. Science 2001; 294:1540–1543.

43 Jego G, Palucka AK, Blanck JP, *et al*. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity 2003; 19:225–234.

44 Santiago-Raber ML, Baccala R, Haraldsson KM, *et al*. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J Exp Med 2003; 197:777–788.

45 Hron JD,Peng SL. Type I IFN protects against murine lupus. J Immunol 2004; 173:2134–2142.

46 Hueber W, Zeng D, Strober S, Utz PJ. Interferon-a-inducible proteins are novel autoantigens in murine lupus. Arthritis Rheum 2004; 50:3239–3249.

47 •• Ng B, Yang F, Huston DP, *et al*. Increased noncanonical splicing of autoantigen transcripts provides the structural basis for expression of untolerized epitopes. J Allergy Clin Immunol 2004; 114:1463–1470.

This study applied an approach using bioinformatics to identify autoantigens with alternatively spliced isoforms. Importantly, these alternatively spliced isoform-specific regions occur within candidate contact sites for antigen presenting molecules and autoantibodies.

48 Latz E, Schoenemeyer A, Visintin A, *et al*. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol 2004; 5:190–198.

49 Leadbetter EA, Rifkin IR, Hohlbaum AM, *et al*. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002; 416:603–607.

50 Marshak-Rothstein A, Busconi L, Rifkin IR, *et al*. The stimulation of Toll-like receptors by nuclear antigens: a link between apoptosis and autoimmunity. Rheum Dis Clin N Am 2004; 30:559–574.

51 Viglianti GA, Lau CM, Hanley TM, *et al*. Activation of autoreactive B cells by CpG dsDNA. Immunity 2003; 19:837–847.