CHAPTER

13

c0013

Origins of antinuclear antibodies

Westley H. Reeves, Yuan Xu, Haoyang Zhuang, Yi Li, Lijun Yang

s0010

INTRODUCTION

p0010

The production of antinuclear antibodies (ANAs) is one of the defining features of SLE. Description of the LE cell phenomenon by Hargraves et al. in 1948 was the first evidence for the existence of these autoantibodies [1]. Close examination of the bone marrow from SLE patients reveals not only the classic LE cell phenomenon (phagocytosis of intact nuclei by mature polymorphonuclear leukocytes in the bone marrow) but also adherence of nuclei to and phagocytosis by less mature myeloid cells (promyelocytes, metamyelocytes, and myelocytes) (Figure 13.1A–C). The description of LE cells was followed by the identification of antinuclear and anti-DNA antibodies in 1957 [2, 3]. The discovery of anti-Sm antibodies by Tan and Kunkel in 1966 provided definitive evidence that autoantibodies in lupus recognize nuclear structures other than

f0010

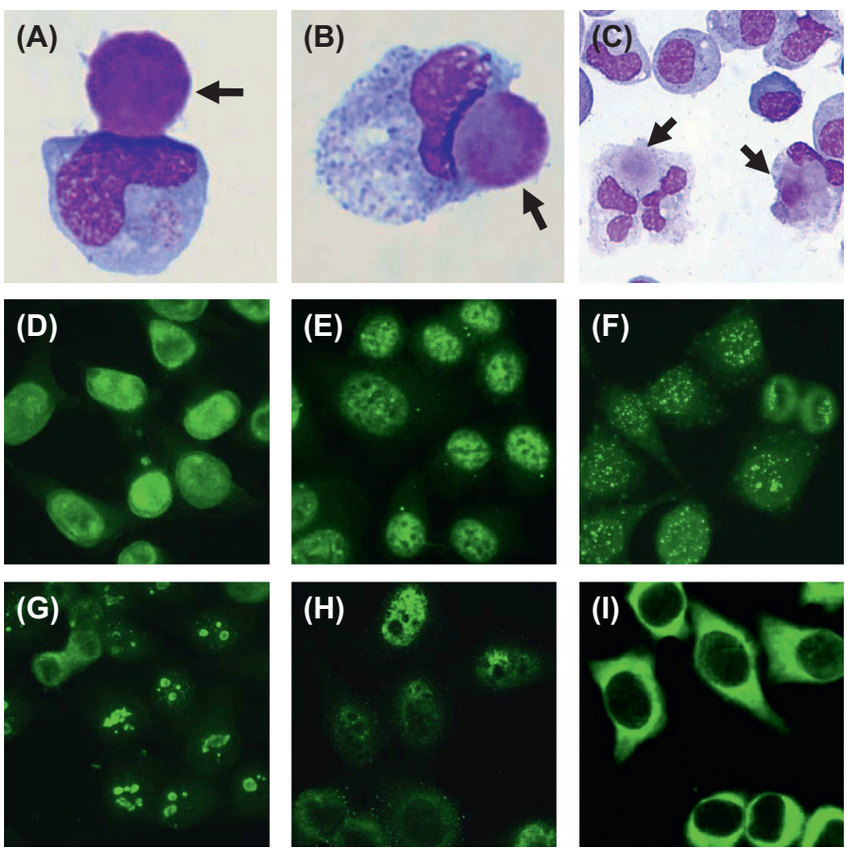


FIGURE 13.1 Detection of antinuclear antibodies. (*A–C*) LE cell phenomenon. Wright-Giemsa staining of a cytospin from a bone marrow aspirate of a 7-year-old girl with lupus nephritis and pancytopenia illustrating granulocytic phagocytosis of astructural nuclear material (arrows) in the bone marrow. (*A, B*) Adherence of nuclei to and phagocytosis of nuclei by immature myeloid cells (*A*, myelocyte and *B*, metamyelocyte, original magnification X1000). (*C*) The "classic" LE cell phenomenon (phagocytosis of nuclei by mature neutrophils) (X1000). (*D–I*) Fluorescent antinuclear antibody test. HeLa cells (human cervical carcinoma cell line) were fixed with methanol and stained with human autoimmune serum at a dilution of 1:40. The binding of antinuclear antibodies to the fixed and permeabilized cells was detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG antibodies. (*D*) Diffuse (homogeneous) pattern produced by anti-double-stranded DNA antibodies; (*E*) fine speckled pattern sparing the nucleolus produced by anti-nRNP antibodies; (*F*) centromere staining exhibited by serum from a patient with CREST syndrome producing anti-CENP-B autoantibodies; (*G*) nucleolar pattern produced by anti-RNA polymerase I autoantibodies from a patient with scleroderma; (*H*) variable nuclear staining pattern produced by autoantibodies against PCNA; (*I*) cytoplasmic pattern produced by serum containing anti-ribosomal P autoantibodies.

© Copyright © 2011 by Elsevier Inc. All rights reserved.

10013-LAHITA-9780123749949

f0015

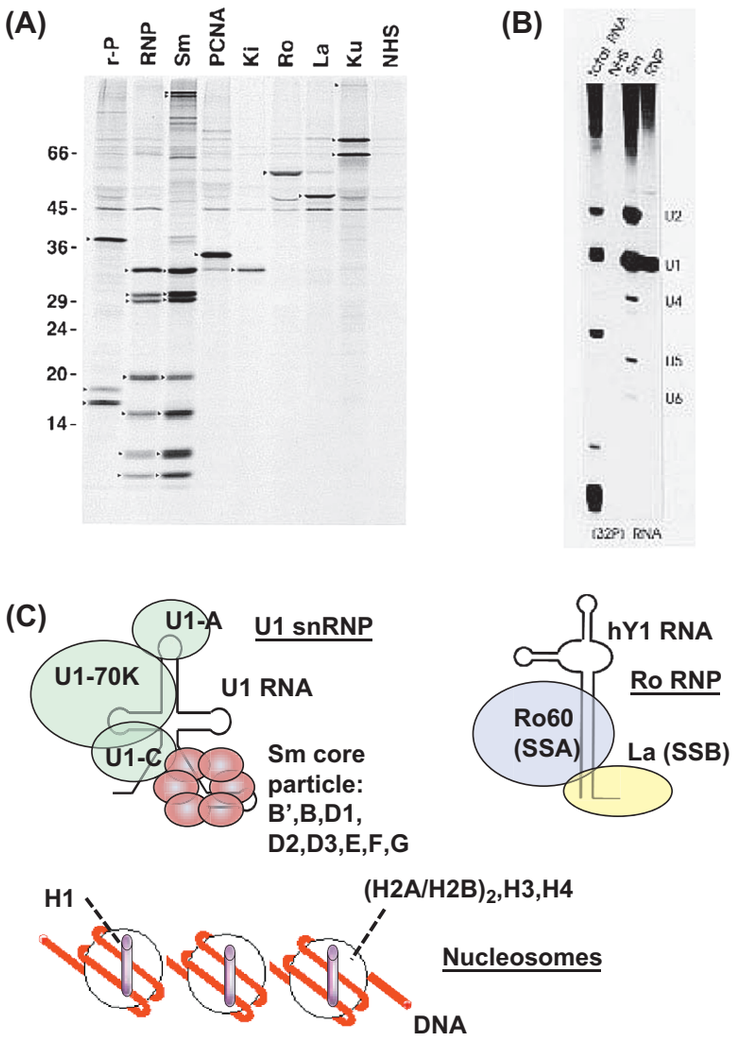


FIGURE 13.2 Antigenic targets of lupus autoantibodies consist of nucleic acid-protein complexes. (*A*) Immunoprecipitation and analysis of [^{35}S]-labeled proteins from K562 cells by SDS-polyacrylamide gel electrophoresis. Immunoprecipitation was performed using prototype sera containing anti- ribosomal P (r-P), nRNP (RNP), Sm, proliferating cell nuclear antigen (PCNA), Ki, Ro (SS-A), La (SS-B), or Ku autoantibodies. Immunoprecipitation with normal human serum (NHS) is shown as a control. Positions of the characteristic protein bands are indicated with arrowheads. Positions of molecular weight standards in kilodaltons are indicated on the left. (*B*) K562 (human erythroleukemia) cells were labeled with [^{32}P] orthophosphate, an extract was immunoprecipitated with anti-Sm or anti-nRNP serum or with normal human serum (NHS). Immunoprecipitates were subjected to phenol extraction, and the radiolabeled RNA was analyzed by gel electrophoresis followed by autoradiography. Positions of U2, U1, U4, U5, and U6 small RNAs are indicated. (*C*) Structure of the U1 small nuclear ribonucleoprotein (U1 snRNP) and Ro ribonucleoprotein (Ro RNP), and nucleosomes. The U1 snRNP is a complex of several proteins (designated 70K, A, B′/B, C, D, E, F, and G) bound to a single molecule of U1 small nuclear RNA. The 70K, A, and C proteins (dark shading) are recognized by anti-nRNP antibodies. The B′/B, D, E, F, and G proteins form a 6S particle termed the Sm core particle. This particle contains the major antigenic determinants recognized by anti-Sm antibodies. The mature hY1 ribonucleoprotein (one of the Ro RNP particles) consists of a single Ro60 (SSA) protein bound to the human Y1 RNA. Newly synthesized hY1 is bound to a second protein, the La (SSB) antigen, which is subsequently cleaved away during maturation of the ribonucleoprotein. Nucleosomes consist of histones H2A/H2B (two copies each) plus H3 and H4 around which DNA is coiled. The DNA between individual nucleosomes is bound to histone H1.

nucleosomes [4], marking the beginning of a 30-year period during which the major autoantibody–autoantigen systems associated with systemic autoimmune diseases were identified and characterized. Over the past 10 years, the pathogenesis of these autoantibodies has been partially elucidated with the discovery that the nucleic acid components of lupus autoantigens are immunostimulatory. This chapter reviews what has been learned about antinuclear antibodies and their molecular targets and pathogenesis.

s0015 DIAGNOSTIC IMPORTANCE OF ANAs

p0015 The autoantibodies produced in SLE are directed primarily against nuclear antigens, most of which are associated with nucleic acids (DNA or RNA). This is the basis for the fluorescent ANA assay, a screening test for SLE that is positive in > 95% of patients [5]. However, the specificity of a positive ANA for SLE is relatively low [6]. ANAs stain cells in a variety of different patterns, corresponding to reactivity with different subsets of nuclear (or cytoplasmic) antigens (Figures 13.1D–I, 13.2A). Some specificities are uniquely associated with SLE (Table 13.1). Anti-dsDNA antibodies, for example, are found in ~70% of SLE patients at some point during their disease, and are 95% specific for the diagnosis [5]. Anti-Sm antibodies are found in ~10–25% of lupus patients' sera depending on ethnicity [7], and also are virtually pathognomonic of SLE [8]. Antibodies to the ribosomal P0, P1, and P2 antigens [9], proliferating cell nuclear antigen (PCNA) [10], and RNA helicase A [11] are highly specific, but less sensitive, markers of the disease (Table 13.1). These "marker" autoantibodies are highly unusual in drug-induced lupus. In contrast, anti-single-stranded (ss) DNA,

10013-LAHITA-9780123749949

t0010 TABLE 13.1 Prevalence of autoantibodies in SLE[a]

	Race/ethnicity			
Autoantibody	**White (n = 789)**	**Black (n = 388)**	**Latin (n = 62)**	**Asian (n = 22)**
dsDNA [b, c]	**21**	**41**	**54**	**39**
Sm[d]	**11**	**41**	**26**	**12**
RNP[d]	12	50	26	18
Ro60 (SS-A)[d]	19	32	37	41
La (SS-B)[d]	6	9	13	5
Su (Ago2)[d]	3	14	19	9
Ribosomal P0, P1, P2[d]	**1**	**4**	**0**	**5**
PCNA[d]	**0.3**	**0.8**	**0**	**0**
Ku[d]	0.6	7	3	0
RNA helicase A[d]	**6**	**3**	**12**	**17**

[a]*University of Florida Center for Autoimmune Disease; patients meeting ACR criteria for the classification of SLE.*
[b]*Crithidia luciliae kinetoplast staining assay. Note that anti-dsDNA antibodies often are present only transiently, but the data are from single serum samples. Estimates in the literature suggest that about 70% of SLE patients will produce anti-dsDNA at some time during their disease.*
[c]***Bold type** indicates autoantibodies that are highly specific for the diagnosis of SLE.*
[d]*Immunoprecipitation assay.*

chromatin/histone, nRNP, Ro-60 (SS-A), La (SS-B), Ro52, Ku, and Su [7], are associated with SLE as well as other systemic autoimmune diseases, but are uncommon in healthy individuals. Remarkably, most of the same antibodies are associated with murine lupus: anti-dsDNA antibodies are produced by (NZB X NZW) F1 mice, anti-dsDNA, Sm, and ribosomal P by MRL mice, and anti-Sm, dsDNA, ribosomal P, and RNA helicase A by mice with pristane-induced lupus [12, 13].

s0020 STRUCTURE OF LUPUS AUTOANTIGENS AND IMPLICATIONS FOR AUTOANTIBODY PRODUCTION

p0020 Although there are thousands of nuclear proteins, only a few are autoantigens in SLE (Table 13.1, Figure 13.2A). These are mainly RNA–protein or DNA–protein complexes comprised of multiple proteins physically associated with nucleic acid (Table 13.2, Figure 13.2). Importantly, the nucleic acid constituents of these antigens are ligands for innate immune system receptors called "Toll-like receptors" (TLRs) 3, 7, 8, and 9 localized within endosomal compartments [14]. Innate immune responses mediated by TLR7 (a receptor for ssRNA) and TLR9 (a receptor for unmethylated CpG motif in dsDNA) are receiving increasing attention as mediators of autoimmune inflammatory disorders, such as SLE (see below and Chapter 17). TLR7 recognition of U1 RNA, a component of the U1 small nuclear ribonucleoprotein (snRNP, recognized by anti-Sm/RNP autoantibodies), and TLR9 recognition of DNA, a component of chromatin (recognized by antihistone/DNA autoantibodies) is linked to the production of type I interferon (IFN-I), which is increased in about 60% of lupus patients (see below and Table 13.2). The structures of some of the major nucleic-acid-containing autoantigens in lupus have been defined by immunoprecipitation studies followed by the analysis of the [^{35}S] methionine/cysteine-labeled proteins and [^{32}P]-labeled nucleic acid components of the complexes recognized by lupus autoantibodies (Figure 13.2A and B, respectively).

Anti-Sm and RNP autoantibodies recognize the U1 ribonucleoprotein (see also Chapter 16) s0025

p0025 The U1 snRNP, an RNA–protein autoantigen, illustrates the general principles applying to many other autoantigen/autoantibody systems (Figure 13.2). It is a macromolecular complex consisting of a group of proteins designated U1-70K, A, B′/B, C, D1/2/3, E, F, and G associated with U1 small nuclear RNA [15] (Table 13.2, Figure 13.2A, B). The U1 snRNP and the U2, U4–U6, and U5 snRNPs play critical roles in RNA splicing from heterogeneous nuclear RNA. The proteins B′/B, D, E, F, and G assemble into a stable 6S particle (the Sm core particle) reactive with anti-Sm, but not anti-RNP, antibodies. Autoantibodies to the Sm core particle are unique to SLE [4, 8]. In contrast, antibodies to the proteins A, C, and 70K, which carry RNP antigenic determinants, may be found in scleroderma, polymyositis, and other subsets of systemic autoimmune disease, as well as SLE. High levels of anti-nRNP antibodies,

10013-LAHITA-9780123749949

t0015 **TABLE 13.2** Protein and nucleic acid components of major lupus-associated autoantigens

Autoantibody	Protein component(s)	Nucleic acid component(s)	TLR recognition
Sm (U1, U2, U4-6, U5 snRNPs)	Sm core particle[a] U1: A, C, 70K[b] U2: A′, B″[b] U4-U6: 150K[b] U5: eight proteins including 200 kDa doublet[b]	 U1 RNA U2 RNA U4-U6 RNA U5 RNA	TLR7
RNP	Sm core particle[a] U1: A, C, 70K[b]	U1 RNA	TLR7
Ro60 (SS-A)	60K Ro, transiently associates with 45K La	hY1, hY3, hY4, and hY5 RNA	TLR7
La (SS-B)	45K La, transiently associates with 60K Ro	RNA polymerase III precursor transcripts	TLR7
Su (Ago2)	Argonaute 2	Micro RNAs	TLR7
Ribosomal P	P0, P1, P2	Ribosomal RNA	?
dsDNA	H2A/H2B, H3, H4	Genomic DNA	TLR9
PCNA	DNA polymerase δ	Genomic DNA	TLR9
Ku/DNA-PK	Ku70, Ku80, DNA-PK_{cs}	Genomic DNA	TLR9
RNA helicase A	150K	Genomic DNA	TLR9

[a]Proteins B′/B, D1/D2/D3, E, F, and G form the 6S Sm core particle (recognized by anti-Sm antibodies), which is shared by all of the U snRNPs listed.
[b]Proteins unique to individual U snRNPs; the U1 snRNP contains three unique proteins, U1-A, U1-C, and U1-70K, which are recognized by anti-RNP antibodies.

without anti-Sm, are seen in mixed connective tissue disease. The U1-A and U1-70K proteins interact directly with U1 RNA via RNA recognition motifs [16]. In addition to the U1 snRNP, other snRNPs, each with a unique uridine-rich (U) RNA species, carry the Sm core particle (Table 13.2). These include the U2, U4/U6, and U5 snRNPs, as well as a number of other less abundant U snRNPs [17]. The U3 ribonucleoprotein is involved in processing of ribosomal RNA and does not carry the Sm/RNP antigenic determinants. The U1, U2, U5, and U4/6 snRNPs are present at levels ranging from 10^6 copies (U1 and U2) to 2×10^5 copies (U5 and U4/U6) per mammalian cell [17]. Along with the Sm core particle, each of these major snRNP particles carries unique protein components (Table 13.2).

s0030 Anti-Ro60 and La autoantibodies recognize cytoplasmic ribonucleoproteins (see also Chapter 15)

p0030 Anti-Ro60 (SS-A), and anti-La (SS-B) autoantibodies are associated with sicca (dry eyes and dry mouth) syndrome, and are not lupus-specific. Anti-Ro60 antibodies are seen in 60–80% of patients with primary Sjogren syndrome and in 10–50% of SLE patients (Table 13.1). Anti-La autoantibodies, which are strongly associated with anti-Ro60, are less frequent. Autoantibodies to the Ro60 (SS-A) antigen bind to cytoplasmic ribonucleoproteins containing a 60-kDa protein associated with a single molecule of human Y1, Y3, Y4, or Y5 RNA (Figure 13.2A, C; Table 13.2). The 47-kDa La (SS-B) antigen is a termination factor for RNA polymerase III that binds transiently to precursors of the Y RNAs. The Ro60 antigen plays an important role in promoting the refolding and/or degradation of damaged (misfolded) U2 and 5S ribosomal RNA molecules [18].

Anti-DNA and nucleosome autoantibodies recognize histone–DNA complexes (see also Chapters 14 and 16) s0035

p0035 Genomic DNA is packaged in histone and non-histone proteins. The DNA is wound around nucleosomes, consisting of two molecules each of H2A/H2B, H3, and H4 plus 145 base pairs of DNA (Figure 13.2C). Between the nucleosomes is "linker" DNA, which is packaged in histone H1. More complex folding leads to the formation of chromatin fibers. Autoantibodies against chromatin and nucleosomes in SLE are diverse, recognizing the H2A–H2B–DNA complex, as well as individual histones, ssDNA, and dsDNA. The LE phenomenon is thought to be mediated primarily by autoantibodies against histone H1, which are exposed when cells undergo necrotic cell death (Figure 13.1A–C) [19]. Of the multiple specificities of antichromatin and nucleosome antibodies, only anti-dsDNA antibodies are specific for the diagnosis of SLE [20]. About 70% of SLE patients have anti-dsDNA antibodies at some time in their disease course, and these autoantibodies are thought to play a role in the

10013-LAHITA-9780123749949

pathogenesis of immune-complex-mediated glomerulonephritis in SLE [21]. The recent discovery that mammalian RNA and DNA interact with TLR7/8 and TLR9, respectively, raises the possibility that immunostimulatory nucleic acids in immune complexes play a role in the pathogenesis of inflammation in SLE by triggering cytokine production.

s0040 Multicomponent autoantigens and epitope spreading

p0040 Lupus autoantibodies frequently occur together as groups of interrelated specificities. Mattioli and Reichlin [22] first reported the strong association of anti-Sm antibodies with anti-RNP. Indeed, sera containing anti-Sm antibodies nearly always contain autoantibodies to the RNP (U1-70K, U1A, and U1C) antigens. Many sera contain autoantibodies to multiple polypeptides, and in one study, only one of 29 sera containing anti-nRNP or Sm antibodies recognized a single protein component of the U1 snRNP, and the majority recognized three or more [23]. Anti-RNP antibodies also are associated with autoantibodies to the U1 RNA component of U1 small ribonucleoproteins in ~40% of sera [24].

p0045 Similarly, anti-Ro (SS-A) and La (SS-B) antibodies are associated with one another [25] and with antibodies to the Y5 small RNA molecule [26], with which both antigens associate, and autoantibodies to DNA and histones (chromatin) are associated with one another. Thus, the macromolecular complexes illustrated in Figure 13.2C appear to be seen by the immune system as units. This is analogous to the immune responses to viral particles, in which T-cell responses against one protein subunit can provide help for antibody responses to other subunits. In the case of influenza, T cells specific for the nucleoprotein help B cells specific for the hemagglutinin [27]. Since B cells can act as antigen-presenting cells, a B cell specific for one component of an autoantigen may internalize an entire complex and present peptides to T cells specific for any of the constituents, potentially explaining the strong associations between autoantibodies specific for the various components of U1 ribonucleoproteins [28]. Consistent with this model of "epitope spreading", mice immunized with recombinant murine La (SS-A) develop anti-La autoantibodies, as well as anti-Ro60. Conversely, immunization with Ro60 causes the production of anti-La as well as anti-Ro antibodies. Epitope spreading also has been reported in antichromatin/nucleosome responses [29].

p0050 In summary, lupus autoantigens typically are multicomponent complexes consisting of both proteins and nucleic acids. Once an immune response is initiated, the physical association of multiple protein and nucleic acid components promotes the spreading of immunity to the other associated components, a phenomenon termed "epitope spreading". As discussed below, the nucleic acid components are immunostimulatory and can signal through TLR7 (RNA) and TLR9 (DNA), perhaps contributing to the selection of autoantigenic targets (see below).

NUCLEIC ACID COMPONENTS OF LUPUS AUTOANTIGENS STIMULATE TYPE I INTERFERON PRODUCTION s0045

Type I interferon and the pathogenesis of lupus autoantibodies (see also Chapter 18) s0050

Interferons, cytokines with antiviral and antiproliferative effects as well as important effects on the activation of immune effector cells, are likely to be involved in the pathogenesis of SLE. They are classified into type I and type II IFNs based on sequence homology, receptor usage, and the cellular origin. IFNγ, the sole type II interferon, is produced primarily by T cells, NKT cells, and NK cells and interacts with a dimeric receptor (IFNGR1/IFNGR2). p0055

The type I interferons (IFN-I), which include multiple IFNα species, IFNβ, and others are produced by leukocytes and fibroblasts and at high levels by plasmacytoid dendritic cells (pDCs) [30]. They all signal via the type I IFN receptor (IFNAR), consisting of two chains, IFNAR1 and IFNAR2, leading to activation of the Janus kinase Jak1 and TYK2 [31]. Jak1/TYK2 then tyrosine-phosphorylate transcription factors of the signal transducer and activator of transcription (STAT) family, including STAT1 and STAT2. p0060

Binding of IFN-I to the IFNAR increases the expression of a group of interferon-stimulated genes (ISGs) regulated by the binding of STAT1 and/or other STATs to a *cis*-acting consensus sequence termed the interferon-stimulated response element (ISRE), which is found in the promoters of all IFN-I inducible genes [31]. ISGs play a key role in the antiviral response and apoptosis. As many as 100 genes are regulated by IFN-I, and the expression of more than 20 of these genes ("interferon signature") is increased in peripheral blood mononuclear cells (PBMCs) from SLE patients, closely reflecting IFN-I levels [32, 33]. Elevated IFN-I expression is associated with severe disease, renal involvement, and autoantibodies against dsDNA and RNA-associated antigens such as Sm/nRNP, SSA/Ro, and SSB/La [34, 35]. Increased IFN-I expression also is seen in lupus skin lesions [36]. IFNα treatment in hepatitis C infection and certain neoplastic diseases can be complicated by autoimmune phenomena, including the induction of ANA and anti-dsDNA antibodies, and there are case reports of overt SLE [37]. A positive ANA test is seen in up to 22% of patients treated with IFNα. In addition, humans with partial trisomy of chromosome 9, which contains p0065

10013-LAHITA-9780123749949

type I interferon gene cluster, over-produce IFN-I and develop anti-RNP and anti-Ro60 autoantibodies [38].

p0070 IFN-I may play a pathogenic role in murine lupus models. In NZB mice, autoimmune hemolytic anemia is milder in the absence of IFN-I signaling [39] and lupus in (NZB X NZW)F1 (NZB/W) mice is accelerated by IFNα [40]. Experimental lupus induced by the hydrocarbon 2,6,10,14-tetramethylpentadecane (TMPD, pristane) is associated with the interferon signature and requires signaling through the IFNAR [41]. Thus, overproduction of IFN-I may be central to the pathogenesis of lupus and autoantibodies characteristic of SLE.

s0055 Cellular sources of IFN-I in SLE

p0075 Although most, if not all, nucleated cells can produce IFN-I, the existence of a minor population of cells in the peripheral blood that produces large amounts of IFN-I was recognized 20 years ago and characterized more recently [30]. These cells are termed "plasmacytoid dendritic cells" (pDC) in view of their eccentrically located nucleus and prominent rough endoplasmic reticulum. In humans, they express CD123 (IL-3 receptor), and HLA-DR, as well as BDCA-2 and BDCA-4. They are $CD11c^-$ in humans, but $CD11c^+$ in mice. Increased production of IFN-I in SLE is attributed to pDCs, a view consistent with the ability of DNA and RNA containing immune complexes to stimulate IFN-I production by pDCs *in vitro* [42, 43]. TLR7 and TLR9 are expressed at high levels on human pDCs and B cells, but not conventional (myeloid) dendritic cells, monocytes, or macrophages. Paradoxically, in contrast to healthy controls pDCs are nearly absent from the peripheral blood of SLE patients [34], possibly due to the activation and migration of pDCs to tissues and/or lymphoid organs [36]. However, there is as yet no direct evidence that the pDCs found in the tissues are responsible for the interferon signature. Moreover, *in vitro* depletion of blood pDCs reduces IFN-I production by only ~40%, suggesting that other cell types are involved [44]. A subset of immature $Ly6C^{hi}$ monocytes is a major source of IFN-I in murine TMPD-lupus [45].

s0060 IFN-I production in murine lupus results from TLR signaling (see also Chapter 17)

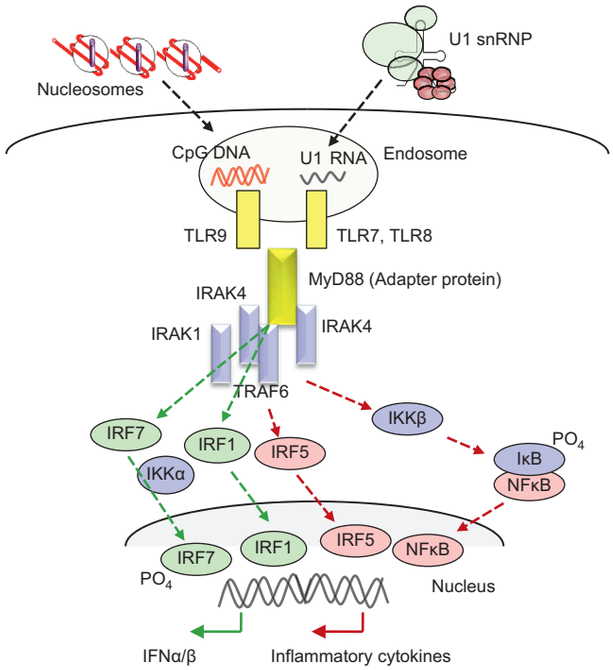
p0080 Several pathways mediate IFN-I production in mammalian cells [14]. TLR3, a sensor for viral dsRNA, and TLR4, a receptor for lipopolysaccharide, both stimulate IFN-I secretion through the adaptor protein TRIF. In contrast, TLR7/8 and TLR9 mediate IFN-I production via MyD88 in response to single-stranded (ss) RNA and unmethylated CpG DNA, respectively. Cytoplasmic receptors that recognize intracellular nucleic acids and induce IFN-I also have been described. Retinoic acid inducible gene-I (RIG-I) and melanoma differentiation associated gene-5 (MDA-5) recognize cytoplasmic RNA and trigger IFN-I by activating IPS-1 and IRF-3, whereas cytoplasmic DNA binds to an intracellular sensor triggering IFN-I production via a TBK-1/IRF-3-dependent pathway [14]. The role of each of these pathways in the pathogenesis of experimental lupus has been examined in the TMPD-lupus model [46]. Autoantibody production and appearance of the interferon signature were unaffected by deficiency of TRIF, IPS-1 or TBK-1, but were abolished by MyD88 deficiency. In addition, anti-Sm/RNP/Su autoantibodies and IFN-I over-expression were unaffected by deficiency of TLR9, but abolished in mice deficient in TLR7 [46], strongly implicating endosomal TLRs in the pathogenesis of lupus autoantibodies.

p0085 There is limited evidence linking abnormal IFN-I production in lupus to exogenous triggers, such as microbial infections, and the bulk of evidence points to endogenous triggers, such as antigens released from dying cells (Figure 13.3). DNA-containing immune complexes can stimulate IFN-I production by normal peripheral blood mononuclear cells (PBMC) [42], and in the presence FcγRIIa (CD32), internalized DNA-containing immune complexes activate human dendritic cells via TLR9 [43]. In mice, chromatin–antichromatin immune complexes (which contain DNA) stimulate dendritic cell activation by engaging TLR9 and FcγRIII and stimulate autoantibody production by antigen-specific B cells by engaging surface immunoglobulin and TLR9 [47, 48]. Similarly, the RNA components of the Sm/RNP antigen (U RNAs) and Ro/SSA antigen (Y RNAs) are TLR7 ligands and stimulate IFN-I production via an endosomal, MyD88-dependent pathway [49–51]. It is likely that endogenous immunostimulatory DNA and RNA molecules originate from apoptotic or necrotic cells [52]. Although Fc receptors are involved in the induction of IFN-I responses *in vitro*, they are dispensable for IFN-I production *in vivo*, at least in the TMPD-lupus model [46]. Interestingly, the clearance of apoptotic cells is impaired in SLE patients (see below), providing a potential source of endogenous nucleic acids. Thus, the interaction of endogenous RNA/DNA ligands with TLRs may be responsible for the "interferon signature" in SLE, although this remains to be verified experimentally.

Signaling from endosomal TLRs s0065

p0090 Mammalian TLRs sense pathogen-associated molecular patterns (PAMPS). Whereas TLRs 1, 2, 4, and 6 are located on the cell surface, TLRs that recognize

10013-LAHITA-9780123749949



f0020 FIGURE 13.3 Nucleic acid components of lupus antigens stimulate IFNα/ß production. Nucleosomal DNA and the small RNA components of ribonucleoproteins, such as the U1 small nuclear ribonucleoprotein (snRNP) are recognized inside of endosomes by TLR9 or TLR7/TLR8, respectively. This leads to the activation of IFNα/ß gene expression via a pathway involving the adapter protein MyD88, several kinases (IRAK1, IRAK4, TRAF6), and the transcription factors interferon regulatory factor (IRF) 7, IRF1, and IRF5. NFκB also is activated. The transcription factors IRF7/IRF1/IRF5 and NFκB activate expression of IFNα/ß and proinflammatory cytokines, such as TNFα, IL-1, and IL-6, respectively.

foreign nucleic acids (TLRs 3, 7, 8, and 9) are located mainly within the endoplasmic reticulum (ER) and/or endosomes [14] (Figure 13.3). TLR3, 7 and 9 are anchored in the endosomal membrane and detect intralumenal nucleic acids after acidification. Subsequently, MyD88 (TLR7, TLR9) or TRIF (TLR3) are recruited, initiating the signaling cascade. Microbial DNA and RNA ligands for TLR7, TLR8, and TLR9 must be released from the organism before interaction with TLRs is possible, and this requires hydrolase enzymes derived from prelysosomes or lysosomes [53]. The steps involved in activation by TLR9 are known in some detail and may be similar for TLR7 [54]. TLR9 is located in the endoplasmic reticulum (ER) of resting dendritic cells, macrophages, and other cell types and is rapidly recruited to early endosomes and subsequently to lysosomes, where it detects unmethylated CpG motifs in DNA [54, 55]. Activation of TLR9 by CpG DNA (and TLR7 by U1 RNA) requires the acidification of late endosomes/lysosomes, and signaling is abolished by inhibitors of endosomal acidification, such as chloroquine (used to treat SLE) or bafilomycin A1 [49].

p0095 An intrinsic ER protein, UNC-93B, is required for innate responses to nucleic acids mediated by TLR 3, 7, and 9 and also is involved in exogenous antigen presentation, providing further evidence that these processes depend on a direct or indirect communication between the ER and endosomes [56]. Interestingly, UNC-93B biases endosomal TLR responses toward DNA and against RNA, and UNC93B deficient DCs are hyperresponsive to TLR7 ligands, but hyporesponsive to TLR9 ligands with no change in TLR3 responses [57].

AUTOIMMUNITY IN MICE WITH DEFECTS IN THE DEGRADATION OF OR RESPONSE TO NUCLEIC ACIDS s0070

p0100 The degradation of nucleic acid–protein autoantigens is influenced by normal pathways of programmed cell death (apoptosis), the uptake of apoptotic cells by phagocytic cells, and the degradation of cellular debris by endolysosomal proteases and nucleases. Thus, there is the potential for improperly degraded cellular nucleic acids to engage endosomal TLRs 3, 7/8, and 9.

Autoimmunity with defects in the Fas-Fas ligand (FasL) pathway s0075

p0105 Apoptosis is mediated by two major signaling pathways, one utilizing death receptors of the TNF receptor family, such as Fas, which involves cysteine proteases (caspases) that degrade proteins and DNA within the dying cell, and a mitochondrial pathway regulated by the anti-apoptotic protein Bcl-2 [58]. Mutations or deficiency of Fas (CD95), its ligand (FasL) and proteins downstream, such as caspase 10, lead to abnormal programmed cell death and are associated with autoimmunity [58]. In mice, deficiency of Fas (*lpr* mutation) or FasL (*gld* mutation) is associated with massive lymphadenopathy, expansion of double-negative ($CD4^-CD8^-$) T cells, and the production of anti-ssDNA and antichromatin autoantibodies in C57BL/6 *lpr* mice. In MRL/*lpr* or MRL/*gld* mice, there is a striking acceleration of lupus-like autoimmune disease, including anti-dsDNA autoantibody production and immune-complex-mediated glomerulonephritis [58]. Unexpectedly, in humans, Fas, FasL, or caspase 10 deficiency also promotes autoimmunity, but usually not lupus. These individuals develop lymphadenopathy, expansion of double-negative T cells, and autoimmune cytopenias (Coombs positive autoimmune hemolytic anemia, autoimmune thrombocytopenia), but rarely develop antinuclear antibodies or clinical manifestations of

10013-LAHITA-9780123749949

lupus. Consistent with defects in which the degradation of DNA is impaired (see below), anti-DNA/chromatin autoantibody production is a prominent feature in *lpr* and *gld* mice (both C57BL/6 and MRL background). It is possible that the failure of caspase 3/7 activation downstream of Fas results in the inability to activate caspase-activated DNase (CAD), a key nuclease involved in internucleosomal DNA cleavage [59], leading to engagement of TLR9 by CpG DNA sequences following the phagocytosis of apoptotic cells.

s0080 Autoimmunity with defects in endosomal DNA degradation

p0110 Along with proteases, acid nucleases (DNases and RNases) are present within phagolysosomes, and complete the process of degrading nucleic acids initiated by CAD. Mice with a mutation in the lysosomal nuclease DNase II, which degrades DNA from apoptotic cells, die *in utero* due to over-production of IFN-I [60]. In contrast, conditional knockouts of DNase II develop rheumatoid-arthritis-like inflammatory arthritis and anti-DNA autoantibodies [61]. Although suggestive of the possibility that the incompletely degraded DNA might stimulate IFN-I production via a TLR, cytokine production is TLR9-independent and the mechanism is unknown.

s0085 Autoimmunity with defects in degradation of misfolded RNA

p0115 Although lysosomal ribonucleases exist, they are poorly studied and knockout mice have not been generated. However, Ro60 knockout mice are deficient in the recognition of misfolded intracellular RNAs, and develop a lupus-like syndrome (antinucleosomal and antiribosomal autoantibodies and glomerulonephritis), suggesting that by promoting the degradation of misfolded and potentially immunostimulatory RNA, Ro60 may protect against the induction of autoimmunity [62]. It is not known whether these mice over-produce IFN-I.

s0090 Autoimmunity with defects in the clearance of apoptotic cells by phagocytes

p0120 Phagocytes express receptors mediating the uptake of apoptotic cells, including complement receptors, CD14, CD36, and scavenger receptor A. Generally, uptake of apoptotic cellular debris by phagocytes is non-inflammatory, whereas the uptake of cells undergoing necrotic death is pro-inflammatory [63]. The C1q and amyloid P molecules are involved in the clearing apoptotic cells and mice deficient in either protein develop lupus-like disease [63]. C57BL/6 mice deficient in the tyrosine kinase MER have a selective defect in the phagocytosis and clearance of apoptotic cells and develop anti-DNA and antichromatin autoantibodies as well as rheumatoid factor [64]. These mice develop only mild renal mesangial changes and proteinuria, and have a normal lifespan, but on a 129Sv background renal disease is more severe. The MER protein associated with GAS6, which can interact with phosphatidylserine exposed on the membrane of cells undergoing apoptosis. TYRO3, AXL, and MER constitute a family tyrosine kinases (TAM receptors) involved in the recognition of apoptotic cells and the suppression of inflammatory responses [65]. Like MER-deficient mice, TYRO3 and AXL knockout mice also develop lupus-like autoimmune disease [63, 65] but anti-dsDNA autoantibody production is most impressive in triple (TYRO3/AXL/MER) knockout mice [66]. Nevertheless, delayed clearance of apoptotic cells, by itself, is insufficient to induce autoantibodies, since CD14 and mannose-binding lectin-deficient mice exhibit defective clearance but do not develop autoimmunity [63].

Autoimmunity with over-expression of TLR7 (see also Chapter 17) s0095

p0125 The ability of TMPD to induce lupus-like autoantibodies and disease is abolished in TLR7-deficient mice [46] and in MRL mice, both TLR7 and TLR9 have been implicated in autoantibody production [67]. Conversely, over-expression of TLR7 promotes lupus. The Yaa (Y-linked autoimmune accelerator) mutation, first identified in male offspring of B6 X SB/Le cross (BXSB mice), is a 4-megabase duplication of the region on chromosome X containing TLR7, which has been translocated to the Y chromosome [68]. Consequently, male BXSB mice have two transcriptionally active copies of the TLR7 gene, whereas females have only one (due to random X-inactivation). The Yaa mutation does not induce lupus on a normal (C57BL/6) background, but greatly accelerates it on the BXSB background as well as in other autoimmune-prone strains, such as NZB/W and B6 FcγRIIb-/- mice, an effect mediated by TLR7 [69]. Male BXSB mice have high levels of anti-dsDNA autoantibodies and an aggressive form of lupus nephritis, abnormalities not seen in BXSB females. TLR7 also is required for the production of autoantibodies against nucleic acids in C57BL/6 mice transgenic for the immunoglobulin heavy and light chains encoding an antibody reactive with RNA, ssDNA, and nucleosomes [70].

p0130 Taken together, these animal models suggest that the production of autoantibodies characteristic of SLE, such as anti-Sm/RNP and anti-DNA, can result from the inability of phagocytes to properly dispose of

10013-LAHITA-9780123749949

nucleic-acid–protein complexes or the enhanced expression of endosomal TLRs, notably TLR7, capable of recognizing cellular nucleic acid.

s0100

ROLE OF T CELLS IN AUTOANTIBODY PRODUCTION (SEE ALSO CHAPTER 7)

p0135 The production of autoantibodies can be dependent or independent of help from $CD4^+$ T cells, and there is evidence for both mechanisms in SLE. T-cell-independent activation of antibody production is stimulated by antigens with repeating epitopes, such as pneumococcal polysaccharide, by TLR ligands, and by B-cell-activating factor of the tumor necrosis factor family (BAFF). T-cell-independent responses generally lead to rapid antibody production by short-lived plasma cells that develop extrafollicularly and produce low-affinity IgM antibodies, although switched isotypes (e.g. IgG) also are produced.

p0140 In contrast, T-cell-dependent autoantibody production implies the involvement of secondary lymphoid organs, such as the spleen or lymph nodes, which provide an optimal milieu for interactions between T cells, B cells, and antigen-presenting cells (APCs). Antigen-activated B cells express the chemokine receptor CCR7 and are attracted to the T-cell zones of secondary lymphoid tissues by the chemokine CCL21 (BLC) [71]. Here they receive help from $CD4^+$ T cells after which they may enter two pathways: (1) migration into follicles with the formation of germinal centers, memory B cells, and long-lived antibody-secreting plasma cells ("follicular pathway") and (2) migration to splenic bridging channels or medullary cords with formation of extrafollicular foci of short-lived plasma cells ("extrafollicular pathway"). In the follicular pathway, B cells (centroblasts) proliferate within the germinal centers and then develop into centrocytes, which upon contacting antigen associated with follicular dendritic cells, can activate $CD4^+$ follicular helper cells (T_{FH} cells). This critical T-cell subset provides signals for the development of memory B cells and long-lived plasma cells, a hallmark of the germinal center reaction. Other features of the follicular (germinal center) pathway include the requirement for CD40–CD40L interactions and the induction of somatic hypermutation of immunoglobulin hypervariable regions and class switch recombination from IgM to IgG, IgA, or IgE [72]. In addition to T_{FH}, other subsets of $CD4^+$ T cells may promote antibody production, including the T_H1, T_H2, and T_H17 subsets.

p0145 Although low-affinity, polyreactive anti-ssDNA autoantibodies produced by B-1 cells bear germline Ig variable region sequences and may be relatively independent of T cell help, much autoantibody production in SLE may be T-cell-dependent [73]. Lupus autoantibodies are skewed toward the T-cell (IFNγ/IFNαβ)-dependent isotypes IgG2a in murine lupus and IgG1 in humans [74]; autoantibody production is decreased in MRL/*lpr* or NZB/W mice treated with anti-CD4 antibodies or CTLA4Ig [75, 76]; and the induction of lupus autoantibodies by TMPD is abolished in T-cell-deficient mice [77]. Serological memory is maintained, at least in part, by long-lived plasma cells [78] thought to be derived mainly from post-germinal center B cells. Additional evidence is the large number of somatic mutations in anti-DNA autoantibody V-regions [79]. Many of the anti-DNA antibodies from MRL/*lpr* mice are members of the same expanded clones [79]. The high frequency of replacement versus silent mutations and their non-random distribution argues that anti-DNA antibodies are selected on the basis of receptor specificity. Thus, the analysis of autoantibody V regions strongly suggests that T cells are involved.

Helper T-cell subsets in autoimmunity s0105

p0150 Upon activation by antigen and an appropriate APC-derived co-stimulatory signal, naïve T cells can differentiate along several pathways (Figure 13.4). In 1986, two subsets of $CD4^+$ T cells, designated T_H1 and T_H2, were defined in mice [80]. Subsequently, other lineages were identified, including the T_H17, T_{FH}, and regulatory T cell (T_{reg}) subsets [81], most of which have been implicated in autoimmunity. Transcriptional programs responsible for establishing these subsets are not stable and the different subsets can interconvert [81].

T_H1 cells s0110

p0155 T_H1 cell differentiation is promoted by IL-12, which induces expression of the transcription factor T-bet, resulting in IFNγ and tumor necrosis factor (TNF) β production (Figure 13.4). Although IFNγ has a major role in macrophage activation, it (along with IFNα/β) also promotes immunoglobulin isotype switching to IgG1 (in humans) and IgG2a (in mice). As most autoantibodies in human lupus are IgG1 (IgG2a in mice), it is thought that T_H1 cells play a significant role in generating lupus autoantibodies. Consistent with that possibility, deficiency of IFNγ decreases autoantibody production (especially of the IgG2a subclass) in mouse models [82–84] and both T_H1 predominance and activation of IFNγ signaling have been reported in human SLE [85, 86].

T_H2 cells s0115

p0160 T_H2 cell differentiation is promoted by IL-4, which induces expression of the transcription factors GATA-3 and STAT6, resulting in IL-4, IL-5, and IL-13 production (Figure 13.4). These cytokines are involved in immune responses to helminths and are instrumental in

10013-LAHITA-9780123749949

f0025

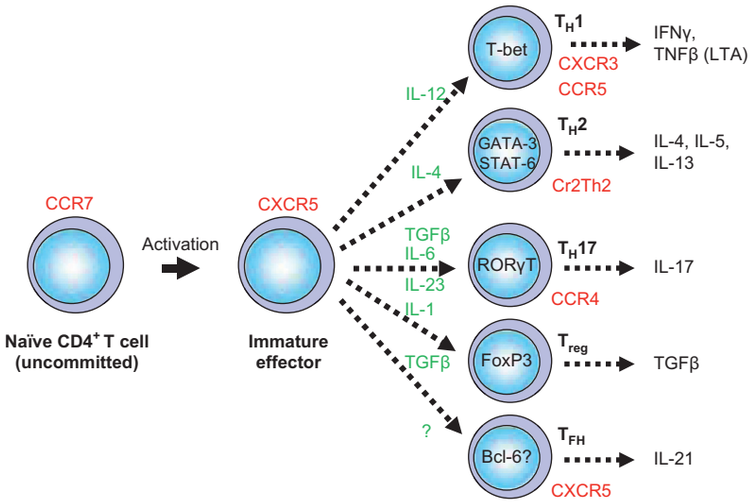


FIGURE 13.4 Development of $CD4^+$ helper T cells implicated in autoantibody production. Upon activation, naïve $CD4^+$ T cells can develop along several pathways in response to polarizing cytokines that induce the expression of specific transcription factors: T-bet in T_H1 cells, GATA-3/STAT-6 in T_H2 cells, RORγT in T_H17 cells, FoxP3 in regulatory T cells (T_{reg}), and Bcl-6 in follicular helper cells (T_{FH}). The individual subsets of mature helper T cells each produce characteristic cytokines that may influence the development of autoimmunity and express chemokine receptors that facilitate homing to specific locations.

promoting antibody responses, especially IgE and also IgG1 (in mice). Despite its importance for immunoglobulin production, there is only limited evidence that IL-4 plays a major role in the pathogenesis of lupus autoantibodies and in the TMPD and BXSB lupus models, IL-4 deficiency actually enhances autoantibody production, presumably by increasing the production of IFNγ [84, 87].

s0120

T_H17 cells

p0165 T_H17 cell differentiation is promoted by TGFβ, IL-6, and IL-23, which induce expression of the transcription factor RORγT, resulting in IL-17 production (Figure 13.4). T_H17 cells may be important mediators of a number of autoimmune diseases, including rheumatoid arthritis in humans and experimental encephalomyelitis in mice. However, their role in the pathogenesis of lupus is just beginning to come into focus [88]. Elevated IL-17 levels in lupus patients correlate with disease activity and levels of anti-DNA antibodies [89, 90]. In the BXD2 mouse model, development of T_H17 cells is enhanced and deficiency of the IL-17 receptor causes defective generation of autoantibodies against DNA and histone [91]. In NZM2328 mice doubly deficient in tumor necrosis factor receptor (TNFR) 1 and 2, anti-dsDNA autoantibody production is enhanced in association with the production of large numbers of T_H17 memory T cells [92]. IL-17 may act synergistically with BAFF to promote the survival, proliferation, and terminal differentiation of B cells in SLE patients [90].

s0125

T_{FH} cells

p0170 The cytokines responsible for T_{FH} cell differentiation and the key transcription factor(s) responsible for IL-21 production have not been identified (Figure 13.4). However, the transcription factor Bcl-6, expressed primarily in germinal center B and T_{FH} cells, may be involved [71]. *Sanroque* mutant mice develop lupus-like autoimmunity with anti-DNA autoantibodies, autoimmune thrombocytopenia, lymphoid hyperplasia, and glomerulonephritis [93]. These mice are deficient in a RING-type ubiquitin ligase (Roquin), causing spontaneous germinal center formation and dysregulation of self-reactive T_{FH} cells expressing high levels of ICOS and IL-21 [93, 94]. Roquin deficiency leads to the production of anti-DNA antibodies reactive in the *Crithidia luciliae* kinetoplast staining assay, but atypical in the absence of IgG2a anti-DNA. Roquin may keep extrafollicular T_{FH} cell development in check, preventing their accumulation. An over-abundance of these cells may provide inappropriate help to autoreactive B cells arising in germinal centers.

s0130

Regulatory T cells

p0175 Regulatory $CD4^+$ T cell (T_{reg}) differentiation is promoted by TGFβ (Figure 13.4). T_{reg} cells express the surface marker CD25 (IL-2Rα) and the transcription factor Foxp3 [95], which controls the development and function of $CD25^+CD4^+$ T_{reg}. $CD4^+$ T_{reg} also are diminished in number and function in SLE, rheumatoid arthritis, and multiple sclerosis [96, 97]. *FoxP3* mutations result in deficiency of $CD25^+CD4^+$ T_{reg}, causing autoimmune/inflammatory disease in mice and humans [95]. Scurfy mice with a FoxP3 mutation develop a fatal multi-organ inflammatory disorder affecting skin, eyes, small intestine, pancreas, and other organs [98]. In humans, FoxP3 mutations result in IPEX (immunodysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, characterized by skin lesions, autoimmune enteropathy, type I diabetes, thyroiditis, chronic inflammation with cytokine production, and autoimmune cytopenias, frequently resulting in death by age 2. However, although autoimmunity is associated with

10013-LAHITA-9780123749949

FoxP3 mutations in both humans and mice, antinuclear antibodies have not been reported. Nevertheless, T_{reg} cells suppress anti-DNA autoantibody production in the NZB/NZW model *in vitro* [99].

s0135 Autoantibody production is affected by peripheral T-cell activation

p0180 Autoreactive T cells are censored through clonal deletion and peripheral inactivation (anergy). In the case of T-cell-dependent immune responses, T-cell tolerance is often a more important mechanism than B-cell tolerance for preventing autoantibody production. The main mechanism for removing autoreactive T cells with high-affinity antigen receptors is clonal deletion in the thymus, whereas tolerance to antigens not expressed in the thymus is maintained by peripheral T-cell inactivation.

s0140 *Importance of co-stimulatory signals*

p0185 Ligation of the T-cell antigen receptor (signal 1) is insufficient, by itself, to activate naïve T cells to proliferate or differentiate and a second co-stimulatory signal (signal 2) delivered by APCs is required [100]. In the absence of a co-stimulation, T-cell receptor occupancy by antigen results in unresponsiveness (anergy) or apoptosis. The best-characterized co-stimulatory molecules are B7-1 (CD80) and B7-2 (CD86), which are expressed on the surface of macrophages, dendritic cells, activated B cells, and other APCs [100]. Delivery of signal 2 along with signal 1 to a naïve T cell alters gene expression, increasing expression of the IL-2 receptor α-chain (CD25), IL-2, and CD40 ligand (CD154). In naïve T cells, the constitutively expressed CD28 molecule is the only receptor for CD80/CD86, but after cross-linking CD28, expression of a higher-affinity receptor, CTLA-4 (CD152), is induced. CTLA-4 inhibits T-cell activation following T-cell receptor ligation [101]. Due to its 20-fold higher affinity for CD80/CD86, CTLA-4 inhibits IL-2 production, limiting the proliferation of activated T cells. It plays a critical role in peripheral tolerance and is expressed on the $CD4^+CD25^+FoxP3^+$ T_{reg} subset [101].

p0190 Additional CD28-like proteins have been identified, including the inducible co-stimulator ICOS, which is a poor inducer of IL-2 but plays a key role in affinity maturation, CD40-mediated class switching, and memory B-cell responses (see T_{FH} cells, above) [102]. Little ICOS is expressed on naïve T cells, whereas it is highly up-regulated on effector/memory T cells, especially T_{FH} cells. ICOS binds to a ligand (ICOSL) expressed on a variety of APCs as well as non-hematopoietic cell types, such as endothelial cells. Humans and mice deficient in ICOS or ICOSL have greatly diminished germinal center responses and are unable to form memory B cells [102].

s0145 *Therapy directed at co-stimulation*

p0195 The regulation of co-stimulatory activity influences susceptibility to autoimmune diseases as illustrated by the development of lupus in Roquin mice, which have increased ICOS expression on their T cells (see above) [94].

p0200 The B7-CD28 co-stimulatory axis has been exploited therapeutically for treating autoimmune diseases, including lupus. CTLA4-Ig, a fusion of the CTLA4 molecule to the Fc portion of immunoglobulin, is a potent inhibitor of co-stimulation and thus T-cell activation. In NZB/W mice, treatment with CTLA4-Ig decreases IgG (but not IgM) anti-dsDNA antibody production and delays the onset of nephritis while prolonging survival [76, 103]. Similarly, in MRL/*lpr* mice, CTLA4-Ig treatment dramatically improves survival, decreases the severity of nephritis, and abolishes IgG anti-dsDNA autoantibody production [104]. In male BXSB mice, treatment with CTLA4-Ig alone does not significantly reduce anti-dsDNA antibody production, but in combination with anti-CD134 (OX40L) monoclonal antibodies, which recognize a surface marker on $CD4^+$ T cells, a reduction of anti-dsDNA antibody production is seen *in vitro* [105]. However, this is associated with a substantial reduction of IL-6 production, raising the possibility that the effect of CTLA4-Ig in this *in vitro* system may be mediated primarily via effects of IL-6 on plasma cell development (see below).

s0150 *Activation-induced T-cell death*

p0205 Most T cells activated in response to foreign antigens ultimately undergo cell death mediated by one of at least two pathways: (1) death receptor-driven apoptosis involving Fas and the TNF receptors, and (2) a Fas-independent mitochondrial pathway involving the Bcl-2 family of proteins [106]. Immunological adjuvants block apoptosis mediated by a third, poorly defined, pathway. Type I interferons, which mediate the adjuvant effect, can rescue T cells from activation-induced death [107, 108], and SLE T cells are resistant to apoptosis [109]. In addition, in mice deficient in IRF-4 binding protein (IBP), T cells are resistant to apoptosis with an accumulation of effector/memory T cells and development of lupus-like autoimmunity [110]. However, although IBP-deficient mice develop anti-DNA antibodies, the overwhelming predominance of IgG1 instead of IgG2a is atypical for lupus.

Summary s0155

p0210 There is strong evidence that T cells are involved in autoantibody production, including indirect evidence from characteristics of the autoantibody response

10013-LAHITA-9780123749949

(e.g. switched isotypes, evidence of affinity maturation), the inability of TMPD to induce autoantibodies in T-cell-deficient mice, the likely involvement of T_H17 and T_{FH} cells in the induction of lupus autoantibodies, and the diminished autoantibody production following CTLA4-Ig treatment. However, there also is evidence that autoantibodies can arise independently of T cells via extrafollicular activation of autoreactive B cells [111].

ROLE OF B LYMPHOCYTES AND PLASMA CELLS IN AUTOANTIBODY PRODUCTION (SEE ALSO CHAPTER 8)

p0215 The two major subsets of B cells, B-1 and B-2, are defined by their surface phenotypes and anatomical distribution [112]. The B-1 subset is enriched in the peritoneal and pleural cavities, and has an IgM^{high}, IgD^{low}, $B220^{high}$, $CD23^{neg}$ phenotype. B-1a cells express moderate levels of CD5, whereas B1-b cells are $CD5^{neg}$. In contrast, the B-2 (conventional) subset is IgM^{low}, IgD^{high}, $B220^{high}$, $CD23^{high}$,$CD5^{neg}$. Most studies suggest that B-1 cells produce polyreactive antibodies, exhibit only limited somatic mutation, develop independently of T cells, and are prone to make low-affinity autoantibodies against repetitive epitopes such as pneumococcal polysaccharide (TI-2 antigens) [112]. In contrast, conventional (B-2) B cells require cognate T cell help and produce high-affinity, somatically mutated antibodies.

B-cell activation and tolerance

p0220 Although B-cell responses to repetitive epitopes are often T-cell-independent, the activation of B cells recognizing low-valency antigens generally requires two signals, one from the antigen receptor and another co-stimulatory signal from the interaction of CD40 on the B cell with CD40 ligand (CD40L) on an activated T cell.

p0225 It has been estimated that as many as half of immature B cells exhibit autoreactivity [113], emphasizing the importance of mechanisms for eliminating or rendering them anergic. Autoreactive B cells are censored by a variety of mechanisms that depend on the presence or absence of T-cell help, the form of the antigen, and the type of B cell. There are differences in the susceptibility of mature vs. immature B cells as well as between B cells responding to T-cell-independent antigens (B-1 and marginal zone subsets) vs. T-cell-dependent antigens (B-2 subset). Deletion (apoptosis), anergy, receptor editing, and immunological ignorance all play a role in determining whether B cells become activated or are tolerized [114].

p0230 Extensive studies of B-cell tolerance to DNA have been carried out in transgenic mouse models [115]. High-affinity anti-dsDNA antibodies are deleted at the pre-B to immature B transitional stage [115] due to the loss of critical homing molecules, such as CD62L, which are important for entry into secondary lymphoid tissues, decreased expression of BAFF, which promotes B-cell survival, and persistent expression of recombination activating genes (RAG) 1 and 2 [114]. Continued expression of RAG1/RAG allows the autoreactive B-cell receptors to be edited by replacement with a different immunoglobulin light chain. Autoreactive B cells that are neither deleted nor modified by receptor editing generally die, but can be rescued by engagement of TLRs on the B-cell surface or by increased BAFF expression [116, 117].

p0235 The germinal center reaction leads to further immunoglobulin diversification through somatic hypermutation (SHM). This can be particularly dangerous, as the potential to generate high-affinity self-reactive immunoglobulins exists and the B cells producing them enter the long-lived plasma cell and memory compartments. The mechanisms by which these cells are regulated may involve deletion due to the lack of T-cell help, competition for BAFF, CD40L, IL-21, ICOS or other co-factors, or inhibitory Fcγ receptor (FcγRIIb) engagement, which inhibits the accumulation of IgG^+ autoreactive plasma cells [114, 118].

p0240 The result is that in most transgenic mouse models, anti-DNA antibody-producing B cells are efficiently censored on non-autoimmune backgrounds [115]. However, in BALB/c mice, anti-DNA B cells that are not deleted can be induced to undergo differentiation into autoantibody-secreting plasma cells when provided with help from $CD4^+$ helper cells or in some cases by TLR7/9 signaling [70]. Conversely, the activation of anti-DNA B cells can be modulated by $CD4^+CD25^+$ regulatory T cells [119].

p0245 In contrast to the relatively tight regulation of autoreactive B cells in BALB/c mice, B cells bearing anti-DNA autoantibody transgenes are not effectively censored on autoimmune backgrounds [115]. For example, follicular exclusion appears to be an important tolerization mechanism for anti-DNA B cells in MRL/*lpr* mice [120]. Recent data suggest that numerous checkpoints involved in limiting autoantibody production can be defective in autoimmune-prone mice.

Maturation of autoreactive B cells

p0250 B cell development can be broadly divided into antigen-independent (bone marrow) and antigen-dependent phases. After high-avidity autoreactive clones are negatively selected in the bone marrow, the surviving B cells are exported to the periphery and there is a divergence of the B-1 and B-2 (conventional) B-cell lineages. Autoreactive B cells can develop in both pathways. However, resting autoreactive B cells do not

10013-LAHITA-9780123749949

secrete immunoglobulin, making the regulation of B-cell differentiation into antibody-secreting plasma cells a key determinant of autoantibody production.

s0175 ***Autoantibody production by B-1 cells***

p0255 B-1 cells develop without T-cell help into plasma cells. The protein tyrosine phosphatase SHP-1 regulates cell numbers in the B-1a compartment. SHP-1-deficient (motheaten viable) mice have a massive expansion of $CD5^+$ B cells and selective IgM hypergammaglobulinemia. In addition to autoantibodies and glomerular immune complex deposition, motheaten mice have a variety of hematological disorders including the accumulation of macrophages and granulocytes in the lungs, causing death at ~9 weeks of age [121] . Their autoimmune syndrome differs significantly from SLE however. Although anti-DNA antibodies reactive on solid phase assays are produced, they do not give nuclear staining on immunofluorescence [121]. Their cytoplasmic staining pattern suggests that they differ in key respects from the typical anti-dsDNA antibodies of SLE. Most evidence suggests that the prototypical lupus autoantibodies, e.g. IgG anti-Sm, are not likely to be derived from B-1 cells.

s0180 ***Autoantibody production by conventional (B-2) cells***

p0260 Upon entering the spleen, conventional B cells mature through the transitional 1 and 2 (T1 and T2) stages (Figure 13.5A) [122]. Some T2 cells home to the splenic marginal zone to become marginal zone B cells, a population characterized by germline immunoglobulin receptors, predominantly IgM isotope, and specificity for TI-2 antigens. However, most T2 cells become recirculating naïve follicular B cells, which undergo further maturation upon receiving cognate T-cell help. Marginal zone B cells, follicular B cells, germinal center B cells, and memory B cells all can develop into plasma cells. In the case of B cells responding to T-cell-dependent antigens, there are two rounds of plasma cell differentiation and antibody production, which take place in different locations within secondary lymphoid tissues (Figure 13.5A). B cells enter the spleen via central arterioles, which are surrounded by T cells (the "periarteriolar

f0030

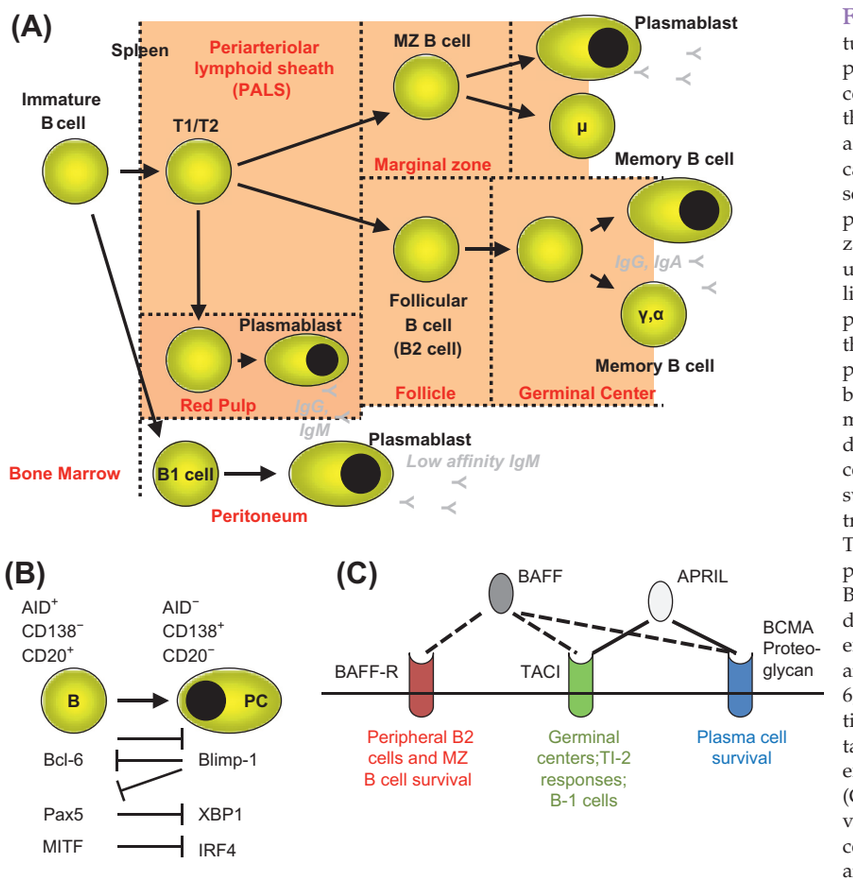


FIGURE 13.5 B-cell development. (*A*) Immature B cells can develop into plasmablasts/plasma cells via several pathways. B-1 cells colonize the peritoneal cavity and can develop there into plasmablasts secreting primarily low-affinity IgM. In contrast, conventional (B-2) B cells can develop into marginal zone B cells that secrete IgM antibodies reactive with T-cell-independent antigens. B-2 cells entering the T-cell zone (the periarteriolar lymphoid sheath) undergo extrafollicular differentiation into short-lived IgG/IgM-secreting plasma cells in the red pulp and also can enter the B-cell follicles, where they may receive T-cell help and develop into proliferating germinal center B cells (centroblasts). The latter can undergo somatic hypermutation and class switching, followed by differentiation into memory B cells and plasma cells (some of them long-lived) that secrete class-switched isotypes such as IgG and IgA. (*B*) Key transcription factors in plasma cell development. The germinal center B-cell transcriptional program is maintained by transcription factors Bxl-6, Pax5, and MITF, which inhibit plasma cell development. Plasma cells develop following the expression of transcription factors Blimp-1, XBP1, and IRF4, which are negatively regulated by Bcl-6, Pax5, and MITF. Conversely, Blimp-1 negatively regulates transcription factors that maintain a B-cell phenotype. The differential expression of characteristic surface markers (CD138, CD20) and intracellular proteins (activation-induced cytidine deaminase, AID) by B cells vs. plasma cells is shown. (*C*) BAFF/APRIL and their receptors. The TNF-like ligands BAFF and APRIL interact with three receptors, BAFF-R, TACI, and BCMA, as indicated. These interactions regulate the survival of various subsets of B cells, as shown below.

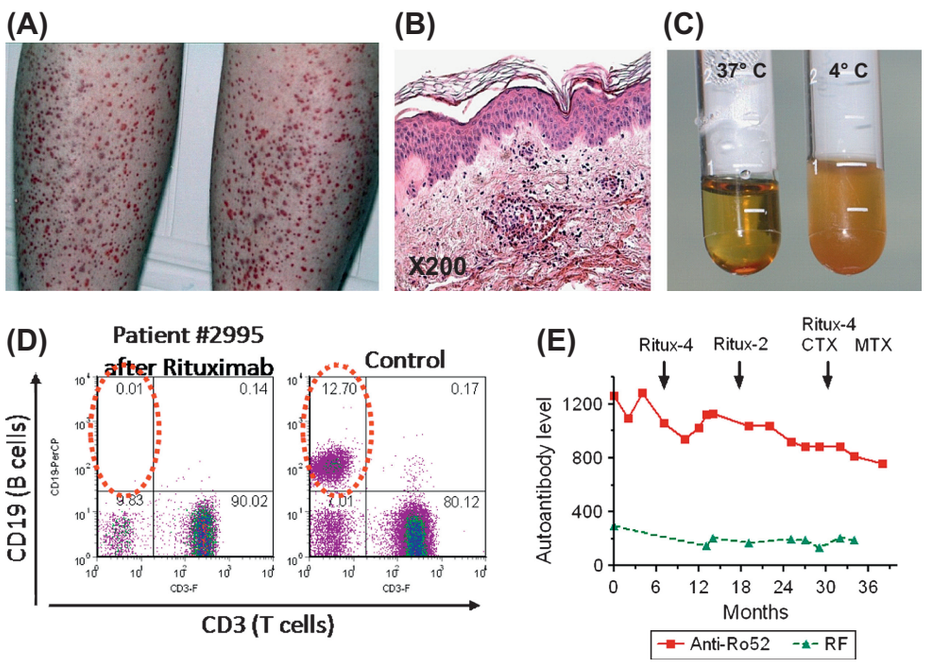
10013-LAHITA-9780123749949

lymphoid sheath"). Antigen-specific B cells receive help from $CD4^+$ T helper cells at the interface of the B- and T-cell zones and after 3–4 days, the activated B cells form extrafollicular foci within the red pulp, leading to the development of short-lived plasma cells (lifespan ~2 weeks). These cells secrete mainly IgM but also can undergo isotype switching. However, their immunoglobulin receptors are unmutated. Subsequently, some of the activated B cells enter primary B-cell follicles and form germinal centers, where they proliferate and undergo somatic hypermutation of their immunoglobulin genes and isotype switching. In general, high-affinity B cells or B cells reactive with repetitive epitopes develop extrafollicularly into plasma cells, whereas low-affinity B cells are directed to germinal centers where they undergo affinity maturation [123]. The germinal center reaction leads to the production of isotype-switched memory B cells and plasma cells (Figure 13.5A), many of which home to the bone marrow and persist for many years as long-lived plasma cells ([78]).

Regulation of plasma cell differentiation

p0265 The gene expression programs of B cells and plasma cells are distinct, and the decision to remain a B cell or undergo terminal differentiation is regulated by several key transcription factors (Figure 13.5B) [122]. The germinal center phenotype is maintained by PAX5, Bcl-6 and other factors whereas Blimp-1, XBP1, and IRF4 expression characterize plasma cell development. Bcl-6 represses Blimp-1 expression and vice versa [122]. Importantly, IL-21 (the product of T_{FH} cells) strongly induces Blimp-1 and is expressed at high levels in male BXSB mice. Cell surface markers characteristic of B cells, such as CD20, are lost as terminal differentiation progresses, whereas new surface markers characteristic of plasma cells, such as CD138, are expressed (Figure 13.5B).

p0270 As the serum half-life of IgG is 1–2 months, the maintenance of antibody levels following immunization must be maintained by continuous antibody secretion. The differentiation of memory B cells into short-lived plasma cells and the generation of long-lived plasma cells are responsible for this "serological memory" [122]. In NZB/W mice, 60% of anti-DNA autoantibody-secreting cells are short-lived and 40% long-lived [124]. Long-lived plasma cells, which can maintain antibody secretion for many years, cannot be eliminated by therapy directed at B-cell-specific markers, such as CD20 (see below and Figure 13.6). The signals regulating whether a plasma cell is



f0035 FIGURE 13.6 Effect of B-cell therapy on autoantibody production. (*A*) Vasculitic lesions on the legs of a 21-year-old woman with Sjogren syndrome complicated by cryoglobulinemic vasculitis. (*B*) Biopsy of one of the skin lesions showing small-vessel vasculitis. (*C*) Serum from the patient stored at 37°C or 4°C, illustrating the formation of cryoprecipitate. (*D*) Depletion of the patient's $CD19^+$ B cells following rituximab (anti-CD20) monoclonal antibody therapy. (*E*) Effect of treatment (Ritux, rituximab; CTX, cyclophosphamide; MTX, methotrexate) on the levels of anti-Ro52 and rheumatoid factor (RF) over a period of 36 months.

10013-LAHITA-9780123749949

short-lived or enters the long-lived compartment are just beginning to come into focus [125]. One factor is B-cell receptor affinity: high-affinity B cells tend to develop into short-lived plasma cells, whereas lower-affinity B cells enter germinal centers and are more likely to become long-lived plasma cells and memory cells. The maintenance of long-lived plasma cells requires continuous expression of Blimp-1 as well as factors provided by so-called "survival niches", especially IL-6 and BAFF produced by stromal cells [126, 127]. In the bone marrow, interactions of BAFF with the BCMA receptor (Figure 13.5C) promote the survival of long-lived plasma cells [128].

p0275 The persistence of long-lived plasma cells, including anti-DNA plasma cells [118], is regulated, in part, by apoptosis mediated by cross-linking of the inhibitory Fc receptor FcγRIIb, which is expressed on plasma cells [129]. Plasma cells accumulate in the bone marrow of FcγRIIb-deficient mice [129], which also develop autoantibodies and glomerulonephritis [130]. Interestingly, plasma cells in NZB and MRL mice do not express significant FcγRIIb and therefore are resistant to apoptosis.

p0280 The two mechanisms for maintaining serological memory (differentiation of memory B cells into short-lived plasma cells and bone marrow long-lived plasma cells) may have important implications for maintaining autoantibody production. Anti-dsDNA autoantibody production is often transient and may be associated with disease activity, consistent with production by short-lived plasma cells. Other autoantibodies, particularly anti-Sm, RNP, Ro, and La, tend to persist at relatively stable levels for many years consistent with their production by long-lived plasma cells.

s0190 Memory B cells

p0285 Besides plasma cells, the germinal center reaction generates long-lived memory B cells, which bear the surface marker CD27 in humans and appear to be maintained without further antigenic stimulation. The B-cell signaling threshold helps regulate the choice between extrafollicular plasma cell and germinal center development as well as whether post-germinal center B cells will become plasma cells or memory B cells [123, 125]. In the absence of complement receptors (CD21/CD35), signaling through the B-cell receptor is impaired, decreasing the generation of plasma cells while having little effect on the generation of memory B cells [131]. Signaling through CD40 also is important, as increased CD40 signaling promotes the development of short-lived plasma cells instead of long-lived plasma cells and memory B cells [125]. Although controversial, memory B cells may play a key role in serological memory due to polyclonal activation by TLR7/9 ligands and the development of short-lived plasma cells [132].

Extrafollicular generation of autoantibodies s0195

p0290 Some autoantibodies, such as anti-DNA and rheumatoid factors, can develop extrafollicularly without T cells [111, 133]. Despite their T-cell independence, they can exhibit significant somatic hypermutation and class switching, features generally thought to typify germinal center reactions [111]. The extrafollicular activation and differentiation of autoreactive cells is MyD88- and TLR7/9-dependent, and is thought to generate primarily short-lived plasma cells [111, 133]. It is unclear whether or not extrafollicular B-cell responses generate memory B cells or long-lived plasma cells and there remains controversy regarding the relative importance of the extrafollicular vs. germinal center pathway in generating lupus autoantibodies.

Effect of B-cell therapy on autoantibody levels (see also Chapter 59) s0200

p0295 Evidence that both long- and short-lived plasma cells are involved in autoantibody production is provided by the experience using pan-B-cell monoclonal antibodies, such as anti-CD20 (rituximab), to treat lupus [134, 135]. In some patients, anti-CD20 therapy dramatically reduces autoantibody levels, but in others there is little effect, probably reflecting the fact that plasma cells are $CD20^-$. Figure 13.6 shows the effect of B-cell depletion therapy in a 21-year-old woman with cryoglobulinemic vasculitis due to Sjogren syndrome, who exhibited purpuric lesions of the legs (Figure 13.6A), a skin biopsy revealing small vessel vasculitis (Figure 13.6B), and the presence of serum cryoglobulins (Figure 13.6C). Treatment with rituximab profoundly depleted her $CD19^+$ B cells, while having no effect on $CD3^+$ T cells (Figure 13.6D). Concomitantly, the vasculitic lesions disappeared, but they recurred approximately a year later and again responded to rituximab. Lesions recurred again after another year, and the patient again was treated successfully with rituximab plus cyclophosphamide; methotrexate was added to prevent further recurrences. Despite the dramatic response of the patient's skin lesions and complete depletion of circulating B cells on three occasions, B-cell therapy had little effect on serum levels of anti-Ro52 autoantibodies or rheumatoid factor (Figure 13.6E), consistent with the possibility that these autoantibodies were produced mainly by long-lived plasma cells. Although therapy directed at pan-B-cell surface antigens is a potentially promising new therapy for autoimmune disease, autoantibody

10013-LAHITA-9780123749949

production (Figure 13.6) and clinical activity are not necessarily improved.

Role of BAFF/APRIL in B-cell maturation and autoantibody production

s0205

p0300 B-cell-activating factor of the TNF family (BAFF, also known as BLyS) and the proliferation-inducing ligand APRIL play an important role in the survival and activation of B cells and plasma cells [136]. These two TNF-family ligands interact differentially with three receptors: BAFF-R (also known as BR-3), TACI, and BCMA (Figure 13.5C). BAFF can bind all three receptors (affinity for BCMA is lower), whereas APRIL binds TACI and BCMA (and heparan sulfate proteoglycans), but not BAFF-R. BAFF and APRIL are expressed by neutrophils and antigen-presenting cells, and are inducible by IFN-I as well as other cytokines [136]. The receptors are found on B-cell subsets: BAFF-R on maturing B cells, TACI on all peripheral B cells and at high levels on B1 and marginal zone B cells, and BCMA on plasma cells. TACI expression is induced by TLR7/9 ligands [137]. BAFF/BAFF-R interactions regulate the differentiation and survival of B-2 and MZ B cells, APRIL regulates CD40-independent class switching and plasma cell survival, and TACI regulates TI-2 responses. TACI-deficient mice develop a lupus-like syndrome characterized by proteinuria, reduced survival, and IgG1 anti-dsDNA autoantibodies [138], but it is unclear whether the more characteristic IgG2a anti-DNA antibodies develop. In contrast, patients with TACI deficiency develop common variable immunodeficiency, but no clinical features of lupus [139].

Lupus-like disease in BAFF transgenic mice

s0210

p0305 BAFF-BCMA interactions are required for the survival of long-lived plasma cells [128], whereas B-cell memory is independent of BAFF/APRIL signaling. BAFF transgenic mice develop hypergammaglobulinemia, autoantibodies, glomerulonephritis, and destruction of the salivary glands [137, 140]. Like lupus patients [141], NZB/W and MRL mice have high levels of circulating soluble BAFF in association with IgG2c (the C57BL/6 equivalent of IgG2a), IgG2b, and IgG3 anti-dsDNA antibodies, rheumatoid factor, and renal disease [137]. Unexpectedly, however, these switched autoantibodies are derived from marginal zone and B-1 cells, and are T-cell-independent. Autoantibody production in BAFF-transgenic mice requires MyD88 and BAFF promotes TLR9-induced isotype switching from IgM to IgG [137]. The production of anti-DNA antibodies is enhanced by TLR4, TLR7, or TLR9 ligand stimulation.

BAFF/APRIL antagonism in the therapy of lupus (see also Chapter 59)

s0215

p0310 In view of the elevated BAFF levels in SLE and the development of lupus-like disease in BAFF transgenic mice, a human monoclonal anti-BAFF antibody (belimumab) that binds to soluble BAFF and inhibits its interaction with BAFF-R, TACI, and BCMA is in clinical trials, and other agents directed against BAFF, APRIL, and BAFF-R are under development [142]. The relative independence of memory B-cell survival from BAFF/APRIL suggests that therapy might not adversely affect recall responses, e.g. to vaccines. Also, long-lived plasma cells are BAFF/APRIL-dependent and therefore should be depleted. To the extent that autoantibody production is maintained by memory B cells, therapy may not abolish the serological abnormalities.

p0315 In a recent phase II clinical trial, belimumab treatment did not significantly reduce disease activity or lupus flares overall [143]. However, in a subset of serologically active patients (defined as ANA titer $\geq$ 1:80 and/or positive anti-dsDNA $\geq$ 30 IU/ml), there was a modest reduction in disease activity. Interestingly, there was a reduction in IgG anti-dsDNA antibodies of 29% vs. 9% for placebo, and a reversion to normal in 15% vs. 3% of placebo-treated controls at 52 weeks. In a preliminary study, anti-Sm and RNP autoantibody levels also appeared to be reduced over a 160-week period (Chatham W, et al. Progressive normalization of autoantibody, immunoglobulin, and complement levels over 3 years of belimumab therapy in systemic lupus erythematosus patients, Poster presentation, PANLAR Congress, Guatemala City, 2008). The relatively low rate of reversion in anti-DNA^+ patients treated with belimumab (15% at 1 year, 30% at 3 years) raises the possibility that much of the anti-DNA response is maintained by memory B cells, which are insensitive to BAFF antagonism.

CD40–CD40 ligand (CD40L) signaling and autoantibody production

s0220

p0320 Strength of the co-stimulatory signal delivered to the B cell via CD40 engagement of CD40L on the surface of activated T cells contributes to autoimmunity. CD40L expression is abnormally regulated in both human and murine lupus [144] . CD40–CD40L interactions are important for generating high-affinity autoantibodies. Neutralizing antibodies to CD40L have been used by themselves or in combination with CTLA-4Ig to treat murine lupus, improving renal disease and decreasing autoantibody production [145, 146]. In SNF_1 lupus mice, anti-CD40 treatment reduces anti-dsDNA and anti-histone/DNA autoantibodies while having no effect on anti-ssDNA or antihistone antibodies and

10013-LAHITA-9780123749949

may block nephritis [147]. In limited clinical trials, anti-CD40L treatment reduced anti-dsDNA antibodies (Farr assay) in one study in which all but one subject was anti-DNA$^+$ [148]; however in a second study in which only 17/85 subjects were anti-dsDNA$^+$, a significant reduction was not noted [149]. Unfortunately, trials in human autoimmune disease were stopped due to thrombotic complications [150].

s0225

FUTURE DIRECTIONS

p0325 Since the previous edition of this book, there has been tremendous progress in our understanding of the origins of lupus autoantibodies. The basis for the autoantigen selectivity in SLE, which is largely restricted to nucleic-acid-binding proteins, now appears to be related to the fact that the nucleic acid components of these antigens are "endogenous adjuvants" capable of interacting with TLR7/8 or TLR9 and stimulating IFN-I production. The importance of IFN-I production stimulated by TLR7/8/9 signaling is suggested in human studies and confirmed in animal models. In addition, the anatomy of autoantibody production in the B-cell follicles, extrafollicular regions, and marginal zones is coming into clearer focus.

p0330 Despite substantial progress, the relative importance of T-cell-dependent vs. -independent B-cell activation in SLE remains unclear. In mouse models, there is evidence for both pathways. Further studies are needed to define to what degree autoantibody production in SLE patients results from cognate T–B interactions and post-germinal center memory/plasma cells and vs. extrafollicular, T-cell-independent (TLR-mediated) responses, a question that may be highly significant for the therapy of SLE. The evidence so far suggests that disruption of T–B interactions (e.g. anti-CD40L, CTLA4-Ig) can partially, but not completely, reduce autoantibody production. Inhibitors of TLR7/8/9 signaling and signaling through the IFN-I receptor are now in clinical trials, and the effects of B-cell depletion therapy and BAFF inhibition are being studied.

p0335 Further studies also are needed to determine how IFN-I enhances autoantibody production: is the main effect an enhancement of activated T-cell survival, upregulation of TLR7/9 expression on anergic/ignorant B cells, an increase in BAFF expression and the survival of plasma cells, or some other mechanism? Finally, it will be of interest to know if autoantibody-producing cells are regulated mainly at the level of tolerance (i.e. the generation and survival of autoreactive B cells) or at the level of plasma cell differentiation – i.e. are there are circulating autoreactive B cells just waiting to undergo differentiation into plasma cells?

s0230

Acknowledgment

p0340 This authors were supported by a research grant AR44731 from NIH/NIAMS.

References

[1] M.M. Hargraves, H. Richmond, R. Morton, Presentation of two bone-marrow elements: the Tart cell and LE cell, Proc. Staff Meetings Mayo Clin. 23 (1948) 25–28.
[2] E.J. Holborow, D.M. Weir, G.D. Johnson, A serum factor in lupus erythematosus with affinity for tissue nuclei, Br. Med. J. 2 (1957) 732–734.
[3] W.C. Robbins, H.R. Holman, H. Deicher, H.G. Kunkel, Complement fixation with cell nuclei and DNA in lupus erythematosus, Proc. Soc. Exp. Biol. Med. 96 (1957) 575–579.
[4] E.M. Tan, H.G. Kunkel, Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus, J. Immunol. 96 (1966) 464–471.
[5] S.M. Edworthy, E. Zatarain, D.J. McShane, D.A. Bloch, Analysis of the 1982 ARA lupus criteria data set by recursive partitioning methodology: new insights into the relative merit of individual criteria, J. Rheumatol. 15 (1988) 1493–1498.
[6] W.C. Shiel, M. Jason, The diagnostic associations of patients with antinuclear antibodies referred to a community rheumatologist, J. Rheumatol. 16 (1989) 782–785.
[7] J. Wang, M. Satoh, F. Kabir, M. Shaw, M.A. Domingo, R. Mansoor, et al., Increased prevalence of autoantibodies to Ku antigen in African-Americans versus Caucasians with systemic lupus erythematosus, Arthritis Rheum. 44 (2001) 2367–2370.
[8] N. Kurata, E.M. Tan, Identification of antibodies to nuclear acidic antigens by counterimmunoelectrophoresis, Arthritis Rheum. 19 (1976) 574–580.
[9] K.B. Elkon, E. Bonfa, N. Brot, Antiribosomal antibodies in systemic lupus erythematosus, Rheum. Dis. Clin. North Am. 18 (1992) 377–390.
[10] Y. Takasaki, D. Fishwild, E.M. Tan, Chraracterization of proliferating cell nuclear antigen recognized by autoantibodies in lupus sera, J. Exp. Med. 159 (1984) 981–992.
[11] Y. Yamasaki, S. Narain, H. Yoshida, L. Hernandez, T. Barker, P.C. Hahn, et al., Autoantibodies to RNA helicase A: a new serologic marker of early lupus, Arthritis Rheum. 56 (2007) 596–604.
[12] A.N. Theofilopoulos, F.J. Dixon, Murine models of systemic lupus erythematosus, Adv. Immunol. 37 (1985) 269–390.
[13] W.H. Reeves, P.Y. Lee, J.S. Weinstein, M. Satoh, L. Lu, Induction of autoimmunity by pristane and other naturally occurring hydrocarbons, Trends Immunol. 30 (2009) 455–464.
[14] T. Kawai, S. Akira, Innate immune recognition of viral infection, Nat. Immunol. 7 (2006) 131–137.
[15] M.R. Lerner, J.A. Steitz, Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus, Proc. Natl. Acad. Sci. USA 76 (1979) 5495–5499.
[16] C.C. Query, R.C. Bentley, J.D. Keene, A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein, Cell 57 (1989) 89–101.
[17] J. Craft, Antibodies to snRNPs in systemic lupus erythematosus, Rheum. Dis. Clin. North Am. 18 (1992) 311–335.
[18] A.J. Stein, G. Fuchs, C. Fu, S.L. Wolin, K.M. Reinisch, Structural insights into RNA quality control: the Ro autoantigen binds misfolded RNAs via its central cavity, Cell 121 (2005) 529–539.
[19] E. Feierl, J.S. Smolen, T. Karonitsch, G.H. Stummvoll, H. Ekhart, C.W. Steiner, et al., Engulfed cell remnants, and not

cells undergoing apoptosis, constitute the LE-cell phenomenon, Autoimmunity 40 (2007) 315–321.
[20] A. Weinstein, B. Bordwell, B. Stone, C. Tibbetts, N.F. Rothfield, Antibodies to native DNA and serum complement (C3) levels: application to diagnosis and classification of systemic lupus erythematosus, Am. J. Med. 74 (1983) 206–216.
[21] D. Koffler, V. Agnello, R. Thoburn, H.G. Kunkel, Systemic lupus erythematosus: prototype of immune complex disease in man, J. Exp. Med. 134 (1971) 169–179.
[22] M. Mattioli, M. Reichlin, Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (Mo) systems in SLE sera, J. Immunol. 110 (1973) 1318–1324.
[23] I.M. Pettersson, M. Hinterberger, T. Mimori, E. Gottlieb, J.A. Steitz, The structure of mammalian small nuclear ribonucleoproteins: identification of multiple protein components reactive with anti-(U1)RNP and anti-Sm antibodies, J. Biol. Chem. 259 (1984) 5907–5914.
[24] R.M. Hoet, P. de Weerd, J.K. Gunnewiek, I. Koornneef, W.J. van Venrooij, Epitope regions of U1 small nuclear RNA recognized by anti-U1RNA-specific autoantibodies, J. Clin. Invest. 90 (1992) 1753–1762.
[25] M. Mattioli, M. Reichlin, Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus: description of a cytoplasmic nonribosomal antigen, Arthritis Rheum. 17 (1974) 421–429.
[26] C. Boulanger, B. Chabot, H. Menard, G. Boire, Autoantibodies in human anti-Ro sera specifically recognize deproteinized hY5 Ro RNA, Clin. Exp. Immunol. 99 (1995) 29–36.
[27] S.M. Russell, F.Y. Liew, T cells primed by influenza virion internal components can cooperate in antibody response to haemagglutinin, Nature (Lond.) 280 (1979) 147–148.
[28] S. Fatenejad, M.J. Mamula, J. Craft, Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles, Proc. Natl. Acad. Sci. USA 90 (1993) 12010–12014.
[29] C. Mohan, S. Adams, V. Stanik, S.K. Datta, Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus, J. Exp. Med. 177 (1993) 1367–1381.
[30] P. Fitzgerald-Bocarsly, J. Dai, S. Singh, Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history, Cytokine Growth Factor Rev. 19 (2008) 3–19.
[31] P.Y. Lee, W.H. Reeves, Type I interferon as a target of treatment in SLE, Endocr. Metab Immune. Disord. Drug Targets. 6 (2006) 323–330.
[32] E.C. Baechler, F.M. Batliwalla, G. Karypis, P.M. Gaffney, W.A. Ortmann, K.J. Espe, et al., Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus, Proc. Natl. Acad. Sci. U. S. A 100 (2003) 2610–2615.
[33] L. Bennett, A.K. Palucka, E. Arce, V. Cantrell, J. Borvak, J. Banchereau, et al., Interferon and granulopoiesis signatures in systemic lupus erythematosus blood, J. Exp. Med. 197 (2003) 711–723.
[34] H. Zhuang, S. Narain, E. Sobel, P.Y. Lee, D.C. Nacionales, K.M. Kelly, et al., Association of anti-nucleoprotein autoantibodies with upregulation of Type I interferon-inducible gene transcripts and dendritic cell maturation in systemic lupus erythematosus, Clin. Immunol. 117 (2005) 238–250.
[35] K.A. Kirou, C. Lee, S. George, K. Louca, M.G. Peterson, M.K. Crow, Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease, Arthritis Rheum. 52 (2005) 1491–1503.
[36] L. Farkas, K. Beiske, F. Lund-Johansen, P. Brandtzaeg, F.L. Jahnsen, Plasmacytoid dendritic cells (natural interferon-alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions, Am. J. Pathol. 159 (2001) 237–243.
[37] L.E. Ronnblom, G.V. Alm, K.E. Oberg, Autoimmunity after alpha-interferon therapy for malignant carcinoid tumors, Ann. Intern. Med. 115 (1991) 178–183.
[38] H. Zhuang, M. Kosboth, P. Lee, A. Rice, D.J. Driscoll, R. Zori, et al., Lupus-like disease and high interferon levels with trisomy of the Type I interferon cluster on chromosome 9p, Arthritis Rheum. 54 (2006) 1573–1579.
[39] M.L. Santiago-Raber, R. Baccala, K.M. Haraldsson, D. Choubey, T.A. Stewart, D.H. Kono, et al., Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice, J. Exp. Med. 197 (2003) 777–788.
[40] A. Mathian, A. Weinberg, M. Gallegos, J. Banchereau, S. Koutouzov, IFN-alpha induces early lethal lupus in pre-autoimmune (New Zealand Black x New Zealand White) F1 but not in BALB/c mice, J. Immunol. 174 (2005) 2499–2506.
[41] D.C. Nacionales, K.M. Kelly-Scumpia, P.Y. Lee, J.S. Weinstein, E. Sobel, M. Satoh, et al., Deficiency of the Type I interferon receptor protects mice from experimental lupus, Arthritis Rheum. 56 (2007) 3770–3783.
[42] H. Vallin, A. Perers, G.V. Alm, L. Ronnblom, Anti-double-stranded DNA antibodies and immunostimulatory plasmid DNA in combination mimic the endogenous IFN-alpha inducer in systemic lupus erythematosus, J. Immunol. 163 (1999) 6306–6313.
[43] T.K. Means, E. Latz, F. Hayashi, M.R. Murali, D.T. Golenbock, A.D. Luster, Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9, J. Clin. Invest 115 (2005) 407–417.
[44] P. Blanco, A.K. Palucka, M. Gill, V. Pascual, J. Banchereau, Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus, Science (Wash. D.C.) 294 (2001) 1540–1543.
[45] P.Y. Lee, J.S. Weinstein, D.C. Nacionales, P.O. Scumpia, Y. Li, E. Butfiloski, et al., A Novel Type I IFN-Producing Cell Subset in Murine Lupus, J. Immunol. 180 (2008) 5101–5108.
[46] P.Y. Lee, Y. Kumagai, Y. Li, O. Takeuchi, H. Yoshida, J. Weinstein, et al., TLR7-dependent and FcgammaR-independent production of type I interferon in experimental mouse lupus, J. Exp. Med. 205 (2008) 2995–3006.
[47] E.A. Leadbetter, I.R. Rifkin, A.M. Hohlbaum, B.C. Beaudette, M.J. Shlomchik, A. Marshak-Rothstein, Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors, Nature (Lond.) 416 (2002) 603–607.
[48] M.W. Boule, C. Broughton, F. Mackay, S. Akira, A. Marshak-Rothstein, I.R. Rifkin, Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes, J. Exp. Med. 199 (2004) 1631–1640.
[49] K.M. Kelly, H. Zhuang, D.C. Nacionales, P.O. Scumpia, R. Lyons, J. Akaogi, et al., "Endogenous adjuvant" activity of the RNA components of lupus autoantigens Sm/RNP and Ro 60, Arthritis Rheum. 54 (2006) 1557–1567.
[50] F.J. Barrat, T. Meeker, J. Gregorio, J.H. Chan, S. Uematsu, S. Akira, et al., Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus, J. Exp. Med. 202 (2005) 1131–1139.
[51] E. Savarese, O.W. Chae, S. Trowitzsch, G. Weber, B. Kastner, S. Akira, et al., U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7, Blood 107 (2006) 3229–3234.

10013-LAHITA-9780123749949

[52] A. Marshak-Rothstein, Toll-like receptors in systemic autoimmune disease, Nat. Rev. Immunol. 6 (2006) 823–835.
[53] C. Watts, Immunology. The bell tolls for phagosome maturation, Science (Wash. D.C.) 304 (2004) 976–977.
[54] E. Latz, A. Schoenemeyer, A. Visintin, K.A. Fitzgerald, B.G. Monks, C.F. Knetter, et al., TLR9 signals after translocating from the ER to CpG DNA in the lysosome, Nat. Immunol. 5 (2004) 190–198.
[55] C.A. Leifer, M.N. Kennedy, A. Mazzoni, C. Lee, M.J. Kruhlak, D.M. Segal, TLR9 is localized in the endoplasmic reticulum prior to stimulation, J. Immunol. 173 (2004) 1179–1183.
[56] K. Tabeta, K. Hoebe, E.M. Janssen, X. Du, P. Georgel, K. Crozat, et al., The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9, Nat. Immunol. 7 (2006) 156–164.
[57] R. Fukui, S. Saitoh, F. Matsumoto, H. Kozuka-Hata, M. Oyama, K. Tabeta, et al., Unc93B1 biases Toll-like receptor responses to nucleic acid in dendritic cells toward DNA- but against RNA-sensing, J. Exp. Med. 206 (2009) 1339–1350.
[58] A. Strasser, P.J. Jost, S. Nagata, The many roles of FAS receptor signaling in the immune system, Immunity 30 (2009) 180–192.
[59] S. Nagata, DNA degradation in development and programmed cell death, Annu. Rev. Immunol. 23 (2005) 853–875.
[60] Y. Okabe, K. Kawane, S. Akira, T. Taniguchi, S. Nagata, Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation, J. Exp. Med. 202 (2005) 1333–1339.
[61] K. Kawane, M. Ohtani, K. Miwa, T. Kizawa, Y. Kanbara, Y. Yoshioka, et al., Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages, Nature (Lond.) 443 (2006) 998–1002.
[62] D. Xue, H. Shi, J.D. Smith, X. Chen, D.A. Noe, T. Cedervall, et al., A lupus-like syndrome develops in mice lacking the Ro 60-kDa protein, a major lupus autoantigen, Proc. Natl. Acad. Sci. U. S. A 100 (2003) 7503–7508.
[63] L.P. Erwig, P.M. Henson, Immunological consequences of apoptotic cell phagocytosis, Am. J. Pathol. 171 (2007) 2–8.
[64] P.L. Cohen, R. Caricchio, V. Abraham, T.D. Camenisch, J.C. Jennette, R.A. Roubey, et al., Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase, J. Exp. Med. 196 (2002) 135–140.
[65] G. Lemke, C.V. Rothlin, Immunobiology of the TAM receptors, Nat. Rev. Immunol. 8 (2008) 327–336.
[66] Q. Lu, G. Lemke, Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family, Science (Wash. D.C.) 293 (2001) 306–311.
[67] S.R. Christensen, J. Shupe, K. Nickerson, M. Kashgarian, R.A. Flavell, M.J. Shlomchik, Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus, Immunity 25 (2006) 417–428.
[68] P. Pisitkun, J.A. Deane, M.J. Difilippantonio, T. Tarasenko, A.B. Satterthwaite, S. Bolland, Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication, Science (Wash. D.C.) 312 (2006) 1669–1672.
[69] J.A. Deane, P. Pisitkun, R.S. Barrett, L. Feigenbaum, T. Town, J.M. Ward, et al., Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation, Immunity 27 (2007) 801–810.
[70] R. Berland, L. Fernandez, E. Kari, J.H. Han, I. Lomakin, S. Akira, et al., Toll-like receptor 7-dependent loss of B cell tolerance in pathogenic autoantibody knockin mice, Immunity 25 (2006) 429–440.
[71] C.G. Vinuesa, S.G. Tangye, B. Moser, C.R. Mackay, Follicular B helper T cells in antibody responses and autoimmunity, Nat. Rev. Immunol. 5 (2005) 853–865.
[72] C.D. Allen, T. Okada, J.G. Cyster, Germinal-center organization and cellular dynamics, Immunity 27 (2007) 190–202.
[73] E.S. Sobel, V.N. Kakkanaiah, M. Kakkanaiah, R.L. Cheek, P.L. Cohen, R.A. Eisenberg, T-B collaboration for autoantibody production in lpr mice is cognate and MHC-restricted, J. Immunol. 152 (1994) 6011–6016.
[74] R.A. Eisenberg, S.Y. Craven, P.L. Cohen, Isotype progression and clonality of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice, J. Immunol. 139 (1987) 728–733.
[75] D. Wofsy, W.E. Seaman, Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4, J. Exp. Med. 161 (1985) 378–391.
[76] B.K. Finck, P.S. Linsley, D. Wofsy, Treatment of murine lupus with CTLA4Ig, Science (Wash. D.C.) 265 (1994) 1225–1227.
[77] H.B. Richards, M. Satoh, J.C. Jennette, T. Okano, Y.S. Kanwar, W.H. Reeves, Disparate T cell requirements of two subsets of lupus-specific autoantibodies in pristane-treated mice, Clin. Exp. Immunol. 115 (1999) 547–553.
[78] A. Radbruch, G. Muehlinghaus, E.O. Luger, A. Inamine, K.G. Smith, T. Dorner, et al., Competence and competition: the challenge of becoming a long-lived plasma cell, Nat. Rev. Immunol. 6 (2006) 741–750.
[79] M. Shlomchik, M. Mascelli, H. Shan, M.Z. Radic, D. Pisetsky, A. Marshak-Rothstein, et al., Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation, J. Exp. Med. 171 (1990) 265–298.
[80] T.R. Mosmann, H. Cherwinski, M.W. Bond, M.A. Geidlin, R.L. Coffman, Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins, J. Immunol. 136 (1986) 2348–2357.
[81] L. Zhou, M.M. Chong, D.R. Littman, Plasticity of CD4+ T cell lineage differentiation, Immunity 30 (2009) 646–655.
[82] C. Haas, M. Le Hir, IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/lpr mice, J. Immunol. 158 (1997) 5484–5491.
[83] D. Balomenos, R. Rumold, A.N. Theofilopoulos, Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice, J. Clin. Invest. 101 (1998) 364–371.
[84] H.B. Richards, M. Satoh, J.C. Jennette, B.P. Croker, W.H. Reeves, Interferon-gamma promotes lupus nephritis in mice treated with the hydrocarbon oil pristane, Kidney Int. 60 (2001) 2173–2180.
[85] M. Akahoshi, H. Nakashima, Y. Tanaka, T. Kohsaka, S. Nagano, E. Ohgami, et al., Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus, Arthritis Rheum. 42 (1999) 1644–1648.
[86] T. Karonitsch, E. Feierl, C.W. Steiner, K. Dalwigk, A. Korb, N. Binder, et al., Activation of the interferon-gamma signaling pathway in systemic lupus erythematosus peripheral blood mononuclear cells, Arthritis Rheum. 60 (2009) 1463–1471.
[87] M.L. Santiago, L. Fossati, C. Jacquet, W. Muller, S. Izui, L. Reininger, Interleukin-4 protects against a genetically linked lupus-like autoimmune syndrome, J. Exp. Med. 185 (1997) 65–70.
[88] L.A. Garrett-Sinha, S. John, S.L. Gaffen, IL-17 and the Th17 lineage in systemic lupus erythematosus, Curr. Opin. Rheumatol. 20 (2008) 519–525.
[89] C.K. Wong, L.C. Lit, L.S. Tam, E.K. Li, P.T. Wong, C.W. Lam, Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity, Clin. Immunol. 127 (2008) 385–393.

10013-LAHITA-9780123749949

[90] A. Doreau, A. Belot, J. Bastid, B. Riche, M.C. Trescol-Biemont, B. Ranchin, et al., Interleukin 17 acts in synergy with B cell-activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus, Nat. Immunol. 10 (2009) 778–785.
[91] H.C. Hsu, P. Yang, J. Wang, Q. Wu, R. Myers, J. Chen, et al., Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice, Nat. Immunol. 9 (2008) 166–175.
[92] N. Jacob, H. Yang, L. Pricop, Y. Liu, X. Gao, S.G. Zheng, et al., Accelerated pathological and clinical nephritis in systemic lupus erythematosus-prone New Zealand Mixed 2328 mice doubly deficient in TNF receptor 1 and TNF receptor 2 via a Th17-associated pathway, J. Immunol. 182 (2009) 2532–2541.
[93] C.G. Vinuesa, M.C. Cook, C. Angelucci, V. Athanasopoulos, L. Rui, K.M. Hill, et al., A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity, Nature (Lond.) 435 (2005) 452–458.
[94] M.A. Linterman, R.J. Rigby, R.K. Wong, D. Yu, R. Brink, J.L. Cannons, et al., Follicular helper T cells are required for systemic autoimmunity, J. Exp. Med. 206 (2009) 561–576.
[95] M. Miyara, S. Sakaguchi, Natural regulatory T cells: mechanisms of suppression, Trends Mol. Med. 13 (2007) 108–116.
[96] M. Miyara, Z. Amoura, C. Parizot, C. Badoual, K. Dorgham, S. Trad, et al., Global natural regulatory T cell depletion in active systemic lupus erythematosus, J. Immunol. 175 (2005) 8392–8400.
[97] X. Valencia, C. Yarboro, G. Illei, P.E. Lipsky, Deficient CD4+CD25high T regulatory cell function in patients with active systemic lupus erythematosus, J. Immunol. 178 (2007) 2579–2588.
[98] H.J. van der Vliet, E.E. Nieuwenhuis, IPEX as a result of mutations in FOXP3, Clin. Dev. Immunol. 2007 (2007) 89017.
[99] N. Iikuni, E.V. Lourenco, B.H. Hahn, C.A. La, Cutting edge: Regulatory T cells directly suppress B cells in systemic lupus erythematosus, J. Immunol. 183 (2009) 1518–1522.
[100] A.J. McAdam, A.N. Schweitzer, A.H. Sharpe, The role of B7 costimulation in activation and differentiation of CD4+ and CD8+ T cells, Immunol. Rev. 165 (1998) 231–247.
[101] R.J. Greenwald, V.A. Boussiotis, R.B. Lorsbach, A.K. Abbas, A.H. Sharpe, CTLA-4 regulates induction of anergy in vivo, Immunity 14 (2001) 145–155.
[102] M.A. Linterman, R.J. Rigby, R. Wong, D. Silva, D. Withers, G. Anderson, et al., Roquin differentiates the specialized functions of duplicated T cell costimulatory receptor genes CD28 and ICOS, Immunity 30 (2009) 228–241.
[103] M. Mihara, I. Tan, Y. Chuzhin, B. Reddy, L. Budhai, A. Holzer, et al., CTLA4Ig inhibits T cell-dependent B-cell maturation in murine systemic lupus erythematosus, J. Clin. Invest. 106 (2000) 91–101.
[104] M. Takiguchi, M. Murakami, I. Nakagawa, I. Saito, A. Hashimoto, T. Uede, CTLA4IgG gene delivery prevents autoantibody production and lupus nephritis in MRL/lpr mice, Life Sci. 66 (2000) 991–1001.
[105] Y.B. Zhou, R.G. Ye, Y.J. Li, C.M. Xie, Y.H. Wu, Effect of anti-CD134L mAb and CTLA4Ig on ConA-induced proliferation, Th cytokine secretion, and anti-dsDNA antibody production in spleen cells from lupus-prone BXSB mice, Autoimmunity 41 (2008) 395–404.
[106] D.A. Hildeman, Y. Zhu, T.C. Mitchell, J. Kappler, P. Marrack, Molecular mechanisms of activated T cell death in vivo, Curr. Opin. Immunol. 14 (2002) 354–359.
[107] A. Le Bon, G. Schiavoni, G. D'Agostino, I. Gresser, F. Belardelli, D.F. Tough, Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo, Immunity 14 (2001) 461–470.
[108] P. Marrack, J. Kappler, T. Mitchell, Type I interferons keep activated T cells alive, J. Exp. Med. 189 (1999) 521–529.
[109] L. Xu, L. Zhang, Y. Yi, H.K. Kang, S.K. Datta, Human lupus T cells resist inactivation and escape death by upregulating COX-2, Nat. Med. 10 (2004) 411–415.
[110] J.C. Fanzo, W. Yang, S.Y. Jang, S. Gupta, Q. Chen, A. Siddiq, et al., Loss of IRF-4-binding protein leads to the spontaneous development of systemic autoimmunity, J. Clin. Invest. 116 (2006) 703–714.
[111] M.J. Shlomchik, Sites and stages of autoreactive B cell activation and regulation, Immunity 28 (2008) 18–28.
[112] K. Hayakawa, R.R. Hardy, Development and function of B-1 cells, Curr. Opin. Immunol. 12 (2000) 346–353.
[113] H. Wardemann, S. Yurasov, A. Schaefer, J.W. Young, E. Meffre, M.C. Nussenzweig, Predominant autoantibody production by early human B cell precursors, Science (Wash. D.C.) 301 (2003) 1374–1377.
[114] C.C. Goodnow, J. Sprent, G.B. Fazekas de St, C.G. Vinuesa, Cellular and genetic mechanisms of self tolerance and autoimmunity, Nature (Lond.) 435 (2005) 590–597.
[115] M.L. Fields, J. Erikson, The regulation of lupus-associated autoantibodies: immunoglobulin transgenic models, Curr. Opin. Immunol. 15 (2003) 709–717.
[116] L. Rui, C.G. Vinuesa, J. Blasioli, C.C. Goodnow, Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling, Nat. Immunol. 4 (2003) 594–600.
[117] M. Thien, T.G. Phan, S. Gardam, M. Amesbury, A. Basten, F. Mackay, et al., Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches, Immunity 20 (2004) 785–798.
[118] H. Fukuyama, F. Nimmerjahn, J.V. Ravetch, The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells, Nat. Immunol. 6 (2005) 99–106.
[119] S.J. Seo, M.L. Fields, J.L. Buckler, A.J. Reed, L. Mandik-Nayak, S.A. Nish, et al., The impact of T helper and T regulatory cells on the regulation of anti-double-stranded DNA B cells, Immunity 16 (2002) 535–546.
[120] L. Mandik-Nayak, S. Seo, C. Sokol, K.M. Potts, A. Bui, J. Erikson, MRL- lpr/lpr mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells, J. Exp. Med. 189 (1999) 1799–1814.
[121] C.M. Westhoff, A. Whittier, S. Kathol, J. McHugh, C. Zajicek, L.D. Shultz, et al., DNA-binding antibodies from viable motheaten mutant mice. Implications for B cell tolerance, J. Immunol. 159 (1997) 3024–3033.
[122] M. Shapiro-Shelef, K. Calame, Regulation of plasma-cell development, Nat. Rev. Immunol. 5 (2005) 230–242.
[123] D. Paus, T.G. Phan, T.D. Chan, S. Gardam, A. Basten, R. Brink, Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation, J. Exp. Med. 203 (2006) 1081–1091.
[124] B.F. Hoyer, K. Moser, A.E. Hauser, A. Peddinghaus, C. Voigt, D. Eilat, et al., Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice, J. Exp. Med. 199 (2004) 1577–1584.
[125] M.J. Benson, L.D. Erickson, M.W. Gleeson, R.J. Noelle, Affinity of antigen encounter and other early B-cell signals determine B-cell fate, Curr. Opin. Immunol. 19 (2007) 275–280.
[126] M. Shapiro-Shelef, K.I. Lin, L.J. McHeyzer-Williams, J. Liao, M.G. McHeyzer-Williams, K. Calame, Blimp-1 is required for

the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells, Immunity 19 (2003) 607–620.
[127] K. Moser, K. Tokoyoda, A. Radbruch, I. MacLennan, R.A. Manz, Stromal niches, plasma cell differentiation and survival, Curr. Opin. Immunol. 18 (2006) 265–270.
[128] B.P. O'Connor, V.S. Raman, L.D. Erickson, W.J. Cook, L.K. Weaver, C. Ahonen, et al., BCMA is essential for the survival of long-lived bone marrow plasma cells, J. Exp. Med. 199 (2004) 91–98.
[129] Z. Xiang, A.J. Cutler, R.J. Brownlie, K. Fairfax, K.E. Lawlor, E. Severinson, et al., FcgammaRIIb controls bone marrow plasma cell persistence and apoptosis, Nat. Immunol. 8 (2007) 419–429.
[130] S. Bolland, J.V. Ravetch, Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis, Immunity 13 (2000) 277–285.
[131] D. Gatto, T. Pfister, A. Jegerlehner, S.W. Martin, M. Kopf, M.F. Bachmann, Complement receptors regulate differentiation of bone marrow plasma cell precursors expressing transcription factors Blimp-1 and XBP-1, J. Exp. Med. 201 (2005) 993–1005.
[132] N.L. Bernasconi, E. Traggiai, A. Lanzavecchia, Maintenance of serological memory by polyclonal activation of human memory B cells, Science (Wash. D.C.) 298 (2002) 2199–2202.
[133] R.A. Herlands, S.R. Christensen, R.A. Sweet, U. Hershberg, M.J. Shlomchik, T Cell-Independent and Toll-like Receptor-Dependent Antigen-Driven Activation of Autoreactive B Cells, Immunity 29 (2008) 249–260.
[134] D.J. DiLillo, Y. Hamaguchi, Y. Ueda, K. Yang, J. Uchida, K.M. Haas, et al., Maintenance of long-lived plasma cells and serological memory despite mature and memory B cell depletion during CD20 immunotherapy in mice, J. Immunol. 180 (2008) 361–371.
[135] J.H. Anolik, J. Barnard, A. Cappione, A.E. Pugh-Bernard, R.E. Felgar, R.J. Looney, et al., Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus, Arthritis Rheum. 50 (2004) 3580–3590.
[136] F. Mackay, P. Schneider, Cracking the BAFF code, Nat. Rev. Immunol. 9 (2009) 491–502.
[137] J.R. Groom, C.A. Fletcher, S.N. Walters, S.T. Grey, S.V. Watt, M.J. Sweet, et al., BAFF and MyD88 signals promote a lupus-like disease independent of T cells, J. Exp. Med. 204 (2007) 1959–1971.
[138] D. Seshasayee, P. Valdez, M. Yan, V.M. Dixit, D. Tumas, I.S. Grewal, Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor, Immunity 18 (2003) 279–288.
[139] U. Salzer, B. Grimbacher, TACItly changing tunes: farewell to a yin and yang of BAFF receptor and TACI in humoral immunity? New genetic defects in common variable immunodeficiency, Curr. Opin. Allergy Clin. Immunol. 5 (2005) 496–503.
[140] F. Mackay, S.A. Woodcock, P. Lawton, C. Ambrose, M. Baetscher, P. Schneider, et al., Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations, J. Exp. Med. 190 (1999) 1697–1710.
[141] J. Zhang, V. Roschke, K.P. Baker, Z. Wang, G.S. Alarcon, B.J. Fessler, et al., Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus, J. Immunol. 166 (2001) 6–10.
[142] M.P. Cancro, D.P. D'Cruz, M.A. Khamashta, The role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus, J. Clin. Invest 119 (2009) 1066–1073.
[143] D.J. Wallace, W. Stohl, R.A. Furie, J.R. Lisse, J.D. McKay, J.T. Merrill, et al., A phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus, Arthritis Rheum. 61 (2009) 1168–1178.
[144] A. Desai-Mehta, L. Lu, R. Ramsey-Goldman, S.K. Datta, Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production, J. Clin. Invest. 97 (1996) 2063–2073.
[145] G.S. Early, W. Zhao, C.M. Burns, Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black x New Zealand white mice, J. Immunol. 157 (1996) 3159–3164.
[146] D.I. Daikh, B.K. Finck, P.S. Linsley, D. Hollenbaugh, D. Wofsy, Long-term inhibition of murine lupus by brief simultaneous blockade of the B7/CD28 and CD40/gp39 costimulation pathways, J. Immunol. 159 (1997) 3104–3108.
[147] C. Mohan, Y. Shi, J.D. Laman, S.K. Datta, Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis, J. Immunol. 154 (1995) 1470–1480.
[148] D.T. Boumpas, R. Furie, S. Manzi, G.G. Illei, D.J. Wallace, J.E. Balow, et al., A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis, Arthritis Rheum. 48 (2003) 719–727.
[149] K.C. Kalunian, J.C. Davis Jr., J.T. Merrill, M.C. Totoritis, D. Wofsy, Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 46 (2002) 3251–3258.
[150] T. Kawai, D. Andrews, R.B. Colvin, D.H. Sachs, A.B. Cosimi, Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand, Nat. Med. 6 (2000) 114.

10013-LAHITA-9780123749949