INTRODUCTION

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...
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.

**DIAGNOSTIC IMPORTANCE OF ANAS**

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,
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].

**TABLE 13.1 Prevalence of autoantibodies in SLE**

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

| 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.

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 [35S] methionine/cysteine-labeled proteins and [32P]-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)

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,
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/U6 snRNPs are present at levels ranging from 10^6 copies (U1 and U2) to 2 x 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).

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

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Protein component(s)</th>
<th>Nucleic acid component(s)</th>
<th>TLR recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm (U1, U2, U4-6, U5 snRNPs)</td>
<td>Sm core particlea</td>
<td>U1 RNA</td>
<td>TLR7</td>
</tr>
<tr>
<td></td>
<td>U1: A, C, 70Kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U2: A', B'b</td>
<td>U2 RNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U4-U6: 150Kb</td>
<td>U4-U6 RNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U5: eight proteins including 200 kDa doubletb</td>
<td>U5 RNA</td>
<td></td>
</tr>
<tr>
<td>RNP</td>
<td>Sm core particlea</td>
<td>U1 RNA</td>
<td>TLR7</td>
</tr>
<tr>
<td></td>
<td>U1: A, C, 70Kb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro60 (SS-A)</td>
<td>60K Ro, transiently associates with 45K La</td>
<td>hY1, hY3, hY4, and hY5 RNA</td>
<td>TLR7</td>
</tr>
<tr>
<td>La (SS-B)</td>
<td>45K La, transiently associates with 60K Ro</td>
<td>RNA polymerase III precursor transcripts</td>
<td>TLR7</td>
</tr>
<tr>
<td>Su (Ago2)</td>
<td>Argonaute 2</td>
<td>Micro RNAs</td>
<td>TLR7</td>
</tr>
<tr>
<td>Ribosomal P</td>
<td>P0, P1, P2</td>
<td>Ribosomal RNA</td>
<td>?</td>
</tr>
<tr>
<td>dsDNA</td>
<td>H2A/H2B, H3, H4</td>
<td>Genomic DNA</td>
<td>TLR9</td>
</tr>
<tr>
<td>PCNA</td>
<td>DNA polymerase δ</td>
<td>Genomic DNA</td>
<td>TLR9</td>
</tr>
<tr>
<td>Ku/DNA-PK</td>
<td>Ku70, Ku80, DNA-PKcs</td>
<td>Genomic DNA</td>
<td>TLR9</td>
</tr>
<tr>
<td>RNA helicase A</td>
<td>150K</td>
<td>Genomic DNA</td>
<td>TLR9</td>
</tr>
</tbody>
</table>

*aProteins 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.

bProteins 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.

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

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
Multicomponent autoantigens and epitope spreading

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].

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].

In summary, lupus autoantigens typically are multi-component 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).
type I interferon gene cluster, over-produce IFN-I and develop anti-RNP and anti-Ro60 autoantibodies [38]. 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 × NZW)F1 (NZB/W) mice is accelerated by IFN-z [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.

### Cellular sources of IFN-I in SLE

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+ monocytes is a major source of IFN-I in murine TMPD-lupus [45].

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

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.

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

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

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 hypersensitive 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**

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

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
Autoimmunity with defects in the clearance of apoptotic cells. 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.

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 that the incompletely degraded DNA might stimulate IFN-I production via a TLR, cytokine production is TLR9-independent and the mechanism is unknown.

Although lyososomal 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.

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 phosphatidylyserine 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)

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].

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

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

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) are also produced.

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 (TFH 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 TFH, other subsets of CD4⁺ T cells may promote antibody production, including the TH1, TH2, and TH17 subsets.

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/IFNβ)-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**

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 TH1 and TH2, were defined in mice [80]. Subsequently, other lineages were identified, including the TH17, TFH, and regulatory T cell (Treg) 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].

**TH1 cells**

TH1 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 TH1 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 TH1 predominance and activation of IFNγ signaling have been reported in human SLE [85, 86].

**TH2 cells**

TH2 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
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].

**TH17 cells**

TH17 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). TH17 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 TH17 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 TH17 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].

**T_FH cells**

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 extracellular 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.

**Regulatory T cells**

Regulatory CD4+ T cell (T_reg) differentiation is promoted by TGFβ (Figure 13.4). T_reg cells express the surface marker CD25 (IL-2Rζ) 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...
Autoantibody production is affected by peripheral T-cell activation

Ligation of the T-cell antigen receptor (signal 1) is insufficient, by itself, to activate naive 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 naive T cell alters gene expression, increasing expression of the IL-2 receptor α-chain (CD25), IL-2, and CD40 ligand (CD154). In naive 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⁺ Treg subset [101].

Additional CD28-like co-stimulatory molecules 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 TFH cells, above) [102]. Little ICOS is expressed on naive T cells, whereas it is highly up-regulated on effector/memory T cells, especially TFH 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].

**Therapy directed at co-stimulation**

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].

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).

**Activation-induced T-cell death**

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**

There is strong evidence that T cells are involved in autoantibody production, including indirect evidence from characteristics of the autoantibody response...
B-cell activation and tolerance

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].

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 autoantibody-secreting plasma cells [114, 118].

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].

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

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

**Autoantibody production by B-1 cells**

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.

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

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 isotype, and specificity for TI-2 antigens. However, most T2 cells become recirculating naive 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

![Figure 13.5](image-url) 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 Bcl-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.
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 (6).

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 TFH 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).

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
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]. 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.

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.

Memory B cells

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

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)

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

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

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 BR3), 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-1 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/APRIL-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

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

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.

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 ≥ 1:80 and/or positive anti-dsDNA ≥ 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

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 SNF1 lupus mice, anti-CD40 treatment reduces anti-dsDNA and anti-histone/DNA autoantibodies while having no effect on anti-ssDNA or anti-histone antibodies and...
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].

**FUTURE DIRECTIONS**

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.

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.

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 circulating autoreactive B cells just waiting to undergo differentiation into plasma cells?

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**References**


ORIGINS OF ANTINUCLEAR ANTIBODIES

I. BASIS OF DISEASE PATHOGENESIS

[230] 13. ORIGINS OF ANTINUCLEAR ANTIBODIES


13. ORIGINS OF ANTINUCLEAR ANTIBODIES


