Structure–function analysis and the molecular origins of anti-DNA antibodies in systemic lupus erythematosus

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Patients with the autoimmune rheumatic disease systemic lupus erythematosus (SLE or 'lupus') develop a wide variety of clinical and serological manifestations including the presence of antibodies to double-stranded DNA (dsDNA), which are often diagnostic and potentially pathogenic. In this review, we have examined the links between the structure and function of anti-dsDNA antibodies, emphasising their clinical associations. We have also reviewed studies involving animal models, the analysis of human antibody sequences and studies of, and using, computer modelling and crystal structure.

Systemic lupus erythematosus (SLE or 'lupus') is a major autoimmune rheumatic disease that is characterised clinically by photosensitive rashes and arthritis; in most cases the kidneys, lungs and central nervous system are affected by the disease. Its clinical diversity is, apparently, matched by its serological diversity (see Table 1; tab001dil). Antibodies [immunoglobulins (Igs) produced by B lymphocytes (B cells)] that are found in patients with SLE appear to target a range of 'self antigens' (host-derived antigens). However, the diversity of the antibodies found in patients with SLE is actually rather narrow, given that a typical mammalian cell contains physiologically significant quantities of as many as 2000 different proteins, which are all potential self antigens (Ref. 1). For example, unlike patients with scleroderma (an autoimmune disease that is characterised by thickening of the skin), patients with SLE do not develop antibodies to the DNA-binding enzyme Scl 70 (also known as topoisomerase 1). Furthermore, the relatively restricted autoantibody profile that is typical of patients with SLE leads to the conclusion that random polyclonal activation of B cells cannot be solely responsible for the production of anti-DNA antibodies (Ref. 2). Precisely which of these SLE-associated autoantibodies are likely to be pathogenic remains to be determined, and represents a considerable challenge.

Anti-dsDNA antibodies

Among the autoantibodies that are present in the serum of patients with SLE, those that bind to dsDNA remain of paramount interest. These antibodies were first identified in the serum of

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Table 1. Summary of the frequency of serum antibodies (and the antigens they are detected with) that are commonly detected in patients with systemic lupus erythematosus (SLE) (tab001dil)

Antigens the Igs bind to (Ab type)[a]	Frequency of patients studied who have detectable antibodies (%): Published studies (worldwide)	Series at the Bloomsbury Rheumatology Unit[b]
Cardiolipin	20–50	34 (for IgG);12 (for IgM)
C1q	20–45	Not studied
dsDNA	40–90	55
Fc IgG (RF)	~25	21
Histone	30–80	Not studied
hsp 70	5–40	5
hsp 90	5–50	25 (for IgG); 35 (for IgM)
La	10–15	13
LAC	10–20	14
'Nucleus' (ANA)	>90	97
RNP	20–35	20
Ro	30–40	32
Sm	5–30	10
ssDNA	≤70	Not studied
Thyroid	≤35	20

[a] For further details about the antigens used, see Table 13 in Ref. 106.
[b] The Bloomsbury Rheumatology Unit is at University College, London, UK.
Abbreviations used: Ab = antibody; ANA = anti-nuclear antibody; C1q = a complement protein; dsDNA = double-stranded DNA; hsp 70 and hsp 90 = two types of human heat shock protein; histone = the protein associated with DNA; Ig = immunoglobulin (antibody); LAC = lupus anti-coagulant; RF = rheumatoid factors, antibodies (usually IgM) that bind to Ig and are produced by patients with rheumatoid arthritis but also with other diseases; ssDNA = single-stranded DNA; Sm, RNP, Ro and La = ribonuclear proteins; thyroid = an extract of human thyroid.

patients with SLE over 40 years ago in four different laboratories (reviewed in Ref. 3). The likely involvement of these antibodies in the pathogenesis of human SLE, and in animal models of SLE, is indicated by (1) the close links between disease 'activity' and levels of anti-dsDNA antibodies in the serum of many, though not all, patients; (2) the elution (removal and collection) of these antibodies from the kidneys of patients with SLE and lupus-prone mice; (3) direct evidence of these antibodies being associated with pathogenicity in isolated rat perfusion systems in which kidneys are dissected from the rat and their function is maintained artificially for a few hours) and mice with severe combined immunodeficiency (SCID); and (4) the fact that although antibodies to single-stranded DNA (ssDNA) are frequently found in the relatives of patients with SLE, those that bind to dsDNA are virtually never detected.

Clinical studies

Many papers published during the past 20 years (reviewed by Spronk and colleagues in Ref. 4) have concluded that levels of anti-dsDNA antibodies [usually measured using enzyme-linked immunosorbent assays (ELISAs) or radioimmunoassays (RIAs)] generally reflect clinical disease activity. This observation appears to be particularly true of renal (kidney) disease, and most of the evidence that anti-dsDNA antibodies are pathogenic has been collected from studies of the kidney. Thus, high levels of anti-dsDNA antibodies, as measured using the Farr assay, which detects high-avidity antibodies (those that bind strongly to their target antigens), and low values of the complement enzyme marker CH50 were found predominantly in patients with lupus nephritis (i.e. who have kidney inflammation associated with SLE; Ref. 5). In contrast, antibodies to ssDNA are not specific for patients with SLE, being present, for example, in many individuals with infectious diseases. Lloyd and Schur (Ref. 6) found that complement depletion and raised titres of anti-dsDNA antibodies were associated more closely with renal than non-renal exacerbations in SLE. Ter Borg and colleagues (Ref. 7), in a prospective

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study of 72 patients, showed that active lupus nephritis was usually associated with high titres of anti-dsDNA antibodies. More recently, Okamura and colleagues (Ref. 8) have demonstrated a close relationship between renal disease activity (assessed by biopsy) and the isotype of Ig (e.g. IgG compared with IgM) produced that bound DNA: activity correlated with IgG reactive against dsDNA but not with IgG reactive against ssDNA or with IgM reactive against either dsDNA or ssDNA. Bootsma and colleagues (Ref. 9), using the concept of a rise in levels of anti-dsDNA antibodies as a means of predicting a clinical relapse, showed that treating such patients with high levels of prednisolone (30 mg/day) reduced the relapse rate, compared with a control group, who were treated with either lower doses of prednisolone or no steroids.

Assessment of disease activity in SLE

A major problem with many of these earlier studies was the unsatisfactory nature of the indices of disease activity that were used to assess SLE. Validated and reliable global-score indices (i.e. score systems in which activity in each system is 'lumped together') have been developed only in the past decade. However, with a disease as diverse as SLE, an activity index that demonstrates 'at a glance' the degree of disease activity in each of the major organs or systems has obvious advantages. The British Isles Lupus Assessment Group (BILAG) has described and validated such a system, based on the 'physician's intention to treat' principle (Ref. 10). Thus, eight organs or systems are distinguished, allowing the easy correlation of activity in a particular organ or system with any serological marker. Using this system in a serial, longitudinal study of 14 Afro-Caribbean patients with SLE, antibodies to dsDNA were shown to correlate with renal disease, cardiopulmonary disease and global scores, but not with musculoskeletal, central nervous system or haematological involvement. Blood samples taken over periods of follow-up in the range of 3–15 years were analysed in this study (Ref. 11).

Historical evidence that anti-dsDNA antibodies can be pathogenic in SLE

Detection alone of anti-DNA antibodies in the kidneys of patients with SLE does not prove such antibodies were responsible for the development of this complication; however, it does place them at 'the scene of the crime'. Koffler and colleagues (Ref. 12) eluted IgG, complement and IgM from the kidneys of patients who had died from lupus nephritis. These eluates possessed anti-nuclear-binding activity; although a specific test for anti-DNA binding was not performed, the anti-nuclear-binding activity of the eluted antibodies could be partially inhibited by the addition of dsDNA, suggesting that at least some anti-dsDNA antibodies were present.

In a study (Ref. 13) of over 40 families affected by SLE (where 21% of 147 relatives had antibodies to ssDNA), only two relatives had antibodies to dsDNA, strongly implicating anti-dsDNA antibodies, but not the anti-ssDNA antibodies, in the disease process (Ref. 14).

Spontaneous disease models of SLE; pathogenicity of anti-DNA antibodies

Some strains of mice spontaneously develop autoimmune disorders that have many of the features that are typical of human SLE (Ref. 15). The most commonly studied strains of mice with murine lupus are: (1) New Zealand black (NZB), (2) BWF1 [NZB x New Zealand white (NZW) F1], (3) MRL/++, (4) MRL-*lpr*/*lpr*, (5) MRL/*lpr*, (6) BXSB, and (7) SNF1 [(NZB) x (SWR) F1] (for a comparison of their features see Table 2; tab002dil). These mice produce elevated levels of total Igs and autoantibodies (such as anti-dsDNA) and are thought to develop nephritis and arteritis as a result of deposition of immune complexes in the kidneys or arteries. Like patients with SLE, these mice present a diversity of disease patterns. In NZB and BWF1 mice, disease occurs primarily in the females. The disease in NZB mice is characterised mostly by a type of haemolytic anaemia that is Coombs positive (with anti-red-cell antibodies produced), and this can be associated with variable production of anti-nuclear antibodies (ANAs). The disease in BWF1 mice is characterised by the production of ANAs and immune-complex-mediated glomerulonephritis (in their kidneys). In MRL/*lpr* mice, the lupus disease more equally affects male and female mice, whereas in BXSB mice, the disease affects males, because of the influence of a Y-linked gene (Ref. 15).

The role of the *lpr* gene in mouse models of SLE

MRL/++ and MRL/*lpr* mice are used as models of SLE, and genetically differ at the *lpr* locus,

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Table 2. Features of disease in lupus-prone mouse models (tab002dil)

Mouse strain; MHC haplotype	Major clinical features	Survival time for 50% of animals in typical group; sex of animal most affected	Major immunological features
New Zealand black (NZB); H-2^{d}	Haemolytic anaemia, glomerulonephritis, lymphomas.	18 months; both sexes affected equally.	Production of anti-erythrocyte antibodies, hyperproduction of IgM, generalised lymphocyte dysfunction.
BWF1 [F1 of (NZB x New Zealand white (NZW)]; H-$2^{d/z}$	Severe immune-complex-mediated nephritis.	7–8 months; females.	Production of anti-nuclear and anti-DNA antibodies, generalised lymphocyte dysfunction.
MRL-*lpr/lpr*, H-2^{k}	Lymphoproliferation, immune-complex-mediated nephritis, rheumatoid arthritis, vasculitis.	2–4 months; females.	Production of anti-nuclear antibodies and rheumatoid factors, proliferation of $Ly1^{+}$ cells[a], generalised lymphocyte dysfunction.
$MRL^{+/+}$; H-2^{k}	As for MRL-*lpr/lpr* but less severe.	18 months; females.	As for MRL-*lpr/lpr* but less severe.
BXSB; H-2^{b}	Haemolytic anaemia, lymphadenopathy, glomerulonephritis.	2–4 months; males.	Production of anti-DNA antibodies, anti-NTA and anti-erythrocyte antibodies, thymic atrophy occurs earlier than normal.
Moth-eaten; H-2^{b}	Hair loss, glomerulonephritis, increased susceptibility to infections.	1 month; both sexes affected equally.	Production of anti-DNA antibodies, anti-NTA and anti-erythrocyte antibodies, immunosuppression.
Palmerston–North; H-2^{q}	Polyarteritis nodosa, immune-complex-mediated nephritis.	11 months; females.	Production of anti-DNA antibodies, hyperactivity of B lymphocytes.
Swan; H-2^{k}	Mild glomerulonephritis.	18 months; both sexes affected equally.	Production of anti-DNA antibodies, thymic atrophy occurs earlier than normal.
SNF1; H-$2^{q/d}$	Severe glomerulonephritis.	4–8 months; females.	Production of anti-DNA and anti-nucleosome antibodies.

[a] $Ly1^{+}$ cells = mouse T lymphocytes (T cells) that have Ly1 antigens on their cell surface, a T-helper subset.
Abbreviations used: H-2 = mouse major histocompatibility complex (MHC) class I; IgM = isotype of immunoglobulin (Ig); NTA = natural thymocytotoxic antibody.

which is mutated in *lpr* mice and is an autosomal recessive gene (Ref. 16). In the homozygous state, *lpr* causes an immunological illness that is characterised by lymphoproliferation and accelerated autoimmunity. MRL/++ mice, which have two copies the wild-type (unmutated) gene at the *lpr* locus, develop an autoimmune syndrome with a much longer survival time of the mice. Congeneic mice on various genetic backgrounds that have two copies of the mutated *lpr* gene (such as C57BL/6-*lpr*/*lpr*, B6-*lpr*/*lpr*, C3H/HeJ-*lpr*/*lpr* and AKR-*lpr*/*lpr*) all develop 'spontaneous' lymphoproliferation and produce autoantibodies. Despite the fact that anti-DNA

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antibodies are produced by, and found in, all of the strains of mice, only MRL/*lpr* mice (with one wild-type and one mutant *lpr* gene) develop severe immune-complex renal disease, which is responsible for the death of these mice by 6–9 months of age. Therefore, the underlying or primary mechanism of autoimmunity in the MRL model lies in the MRL/++ background, and the *lpr* gene appears to act by accelerating and increasing the severity of the disease.

The *lpr* gene is now known to encode a defective configuration of the Fas receptor (Ref. 17), the normal form of which mediates a signalling pathway that initiates apoptosis (programmed cell death; Ref. 18). Fas is a cell-surface protein that plays a major role in the induction of apoptosis in lymphoid cells. Mice with mutations in *lpr* and/or lpr^{cg}, the gene(s) encoding Fas in mice (Ref. 19), are characterised by prolonged survival of B cells and other features that are similar to those found in patients with SLE, suggesting that in these animals interference with apoptosis might be important in the pathogenesis of their autoimmune disease (see below). Because of the involvement of *lpr* in apoptosis, which is important to T-cell function, we recently investigated whether MRL/*lpr* mice had increased activity in the T-helper (Th) subset of T cells (Ref. 20). T cells in the lymph nodes (of both mice and humans) produce soluble factors that enhance B-cell activation and, in turn, cause the accumulation of large numbers of lymphocytes in the spleen and lymph nodes. These T cells are either conventional $CD4^+$ T cells or $CD4^-$ $CD8^-$ T cells (also known as 'double negative' T cells). In MRL/*lpr* mice, a failure of apoptosis of $CD4^+$ T cells in the periphery of the thymus allows self-reactive T cells to persist and ultimately to drive autoantibody production by B cells (Ref. 21). Our studies revealed no significant difference in the extent of apoptosis in the organs of MRL/*lpr* mice compared with normal (non-autoimmune) BALB/c mice at one month of age. At this age, the MRL/*lpr* mice had low levels of serum anti-ssDNA antibodies and anti-dsDNA antibodies, and showed no detectable impairment in renal functions. This observation confirms that in MRL/*lpr* mice, at one month, when the distribution of apoptotic cells was normal, there was no detectable lupus disease. Initially, in the MRL/*lpr* mice, there were no global defects in the negative selection of the T-cell repertoire, and the positive selection of subsets of $CD4^+$ T cells and $CD8^+$ T cells appeared to be normal (Ref. 22). Failure of apoptosis of self-reactive $CD8^+$ T cells can then lead to downregulation of CD8 and persistence as $CD4^-$ $CD8^-$ T cells, which contribute to the lymphadenopathy. It should be noted that in the MRL/*lpr* mice, the size of the thymus increased in parallel with the augmented activity of the Th T cells (Ref. 23).

Anti-DNA antibodies in mice

In mice, the genetic basis of the ability to produce anti-dsDNA antibodies has not been completely defined. In most strains, nephritis is associated with the co-inheritance of several genes including major histocompatibility complex (MHC) genes. In the SNF1 mouse, nephritis is linked to the $I\text{-}A^q$ locus of the normal SWR parent, whereas in BWF1 mice, it is linked to the $I\text{-}E^b$ chain from the NZW parent, a different MHC gene.

Each of these autoimmune mouse strains has a polyclonal B-cell hyperactivity (many clones of B cells are affected) that causes hypergammaglobulinaemia (high levels of IgG antibodies) and an increased number of antibody-forming cells, including those that produce antibodies that bind to nuclear antigens. Although the B cells that are capable of producing the high-affinity anti-dsDNA antibodies are present in normal mice, they do not proliferate and actually produce such antibodies. Experimentally, to investigate the relationship between autoimmunity and Fas-mediated apoptosis (see below), using appropriate fusion-partner cells (Ref. 24), autoantibody-producing cells can be 'immortalised' to produce Igs in culture.

Control of anti-DNA antibodies in mice by T cells and cytokines

T cells probably play an important role in these mouse models of SLE because experimental elimination of Th T cells in SNF1 mice virtually prevented disease, and the removal of Th cells in BWF1 mice also improved disease outcome (Ref. 25). $CD4^+$ T cells, which can induce B cells to secrete cationic IgG anti-dsDNA antibodies, have been cloned from SNF1 mice, which develop an SLE-like autoimmunity (Ref. 26). Studies on the role of those T cells that express CD40 ligand (CD40L) in the development of glomerulonephritis in SNF1 mice have shown that blocking the interaction between CD40L on the pathogenic Th cells and CD40 on the lupus B cells,

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