Humoral autoimmunity in systemic rheumatic disease
A review

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ABSTRACT – ‘Antinuclear antibody’ is a term now encompassing more than a dozen specificities, and cheap tests for these autoantibodies are readily available. Taken together with the clinical picture the tests can help in the fine tuning of diagnosis and perhaps prognosis within the connective tissue diseases. Certain anticytoplasmic antibodies appear to identify particular disease subsets and overlap syndromes, and their detection in conditions such as congenital heart block and recurrent abortion points to the presence there of autoimmune mechanisms. The antibody–disease relationships are reviewed here and the underlying mechanisms are explored. Associations between antibodies themselves and the paucity of evidence for direct pathogenetic effects in many cases leave open the possibility that antinuclear antibodies are clues to aetiology, reporters of a past event initiating both disease and autoantibody production. Retroviruses are candidates fast coming under scrutiny. Arcane though names such as Ro, La, Sm and Jo-1 may appear, much is now known about the intracellular targets of the antibodies; most are enzymes or particles active in DNA replication and the synthesis of RNA and protein. Hence, autoantibodies are useful tools for the molecular biologist as well as the clinician. New knowledge about autoantibodies may yield insights into the aetiology, as well as the pathogenesis, of systemic rheumatic diseases.

Advances in autoantibody research are shedding new light on the connective tissue diseases. The antibodies to cellular antigens are relatively few in number and their origin remains a mystery, yet they are proving of value in management and the recognition of new syndromes.

We all harbour the ability to make autoantibodies against our own cells and tissues. Low levels may serve naturally to accelerate the removal of cellular debris by the reticuloendothelial system [1], and some autoantibodies have close structural relationships with antibodies to bacteria. In the connective tissue diseases, the checks and balances are disturbed, and autoantibodies reach high titres, accounting for a considerable proportion of the circulating immunoglobulin. Yet this is no release of the flood-gates. Just a few autoantibodies are synthesised in abundance in each condition and this selectivity makes it improbable that these are random responses to tissue damage. Rather, we hope autoantibodies are a clue to aetiology, reporters of some past hijack of cells by a pathogenic virus. Such autoantibodies may then also damage tissues and contribute to the resulting syndrome, or may play no further part at all. At any rate, whether a consequence of the peculiar way an antibody could damage the body or an enigmatic clue to aetiology, the remarkable disease specificity is the basis of the clinical value of autoantibody testing. This review concerns some of the newer antibodies to cellular antigens found chiefly in autoimmune rheumatic and hepatic disease [2]. Organ-specific autoimmunity (to thyroid and pancreatic islet cells, for instance) is excluded. The clinician’s request may be precise—for antibodies to DNA or cardiolipin, perhaps—but, when he asks for ‘ENA’, a whole series of antibodies is involved; what follows may help with their interpretation.

Historical

Old Balloney (1642) distinguished rheumatism (the flowing of humours) from gout (the dripping of humours), and Sydenham described rheumatic fever in 1685, yet classification of rheumatic disease is still almost as difficult as in Heberden’s day (1802): ‘The rheumatism is a common name for many aches and pains, which have yet got no peculiar appellation, though owing to very different causes. It is besides often hard to be distinguished from some which have a certain name and class assigned to them’.

Studies of the blood have proven a fruitful approach to this problem. First came Garrod’s murexide test for uric acid (1848), then the Wassermann reaction (1906) with false positive tests first noted in SLE in the 1940s. The war years saw also the recognition of the LE cell phenomenon by Hargraves and rheumatoid factor by Rose and by Waaler. Recognition of the LE cell phenomenon as an opsonin reaction involving antibody to nucleoprotein led to the immunofluorescence technique for antinuclear antibody (Friou; Holborow et al., 1957), the description of various ANA patterns (Beck, 1960), the detection of antibodies to...
several soluble cellular antigens by immunodiffusion (Anderson et al., 1961; Tan, 1966; Reichlin, 1972) and the Farr assay for antibody to DNA (1969). Assays for antibodies to phospholipids and neutrophil cytoplasm are the newcomers of the 1980s.

Antinuclear antibody

The ANA is a simple screening test for autoimmune disease, but for every positive result in a case of systemic lupus erythematosus many more positives will arise because of drug therapy, old age, chronic infection, rheumatoid arthritis, or another autoimmune disease. At least 5% of patients with SLE are ANA-negative, as are about a quarter of patients with other connective tissue diseases. Many of these ANA-negative cases can be picked up by one of the tests described below.

High titres of ANA are more likely to be significant, and the pattern of ANA staining may be helpful. This is particularly so when large tissue culture cells are used as the substrate for immunofluorescence rather than frozen tissue sections. Homogeneous staining is frequent in SLE, drug-induced lupus and chronic active hepatitis, and this type of ANA gives rise to the LE cell phenomenon. Speckled staining is perhaps more frequent in overlap syndromes, and nucleolar staining may suggest scleroderma. However, several antibodies give similar staining patterns, and such results are only useful in conjunction with the whole clinical picture.

Anti-centromere antibody may be an exception. Recognised by its speckled staining of chromosomes in tissue culture cells, it is found almost solely in patients with severe Raynaud’s phenomenon related to a relatively benign form of scleroderma known as CREST syndrome (calcinosis, Raynaud’s phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia) [3]. Thus the finding of this antibody indicates a relatively good prognosis in systemic sclerosis but a guarded one in a patient with Raynaud’s syndrome.

LE cells

The LE cell [4] is a neutrophil leukocyte that has phagocytosed the nucleus of a dead cell. The test depends on opsonisation of the nuclear material by homogeneous-pattern ANA (antibody to DNA and histones). Anti-DNA assays have superseded it in the diagnosis of SLE.

Anti-DNA antibodies and systemic lupus erythematosus

Antibodies to double-stranded DNA usually indicate a diagnosis of SLE, but positive results occasionally arise in rheumatoid arthritis, chronic active hepatitis, systemic sclerosis and chronic infections such as malaria. In drug induced lupus the rise is usually slight except in the case of penicillamine. Only about 70% of patients with SLE are positive even after a long period of observation (partly because the normal range has to be set quite high to exclude non-specific results in other conditions). A sudden rise or fall in titre sometimes heralds a flare, and complement-fixing anti-DNA antibodies may cause nephritis, but there are so many exceptions that decisions about treatment are best made on clinical grounds, avoiding the temptation to keep up steroids just because the test result is high [5].

Antiphospholipid antibodies, thrombosis and abortion

Phospholipids are an important constituent of cell membranes, including platelets and blood vessel walls. High levels of antiphospholipid antibody are associated with thrombosis and recurrent abortion [6]. It is now thought that the antibody exaggerates platelet aggregation by a direct effect on platelets rather than by inhibiting the release of prostacyclin from vascular endothelium [7]. First discovered as the false positive Wassermann reaction and then as the lupus anticoagulant (a paradoxical term based on the prolongation of clotting tests in vitro), the measurement of antiphospholipid (or ‘anticardiolipin’) antibodies is now becoming widely available by the ELISA and radioimmunoassay techniques. Raised levels of antibody are found in 40% of patients with SLE, but it is chiefly those with the top 10% titre and more often those with IgG antibodies who are at greatest risk of complications such as deep venous thrombosis, pulmonary embolism, renal vein thrombosis, Budd–Chiari syndrome (rare), premature stroke, myocardial infarction, mesenteric ischaemia, pulmonary hypertension, and placental insufficiency resulting in abortion [8].

Often there are other clues to the diagnosis of this thrombotic syndrome. The platelet count may be reduced to around 60–100 × 10⁹/litre, and hypertension (sometimes labile) and livido reticularis are common, at least in patients prone to arterial thrombosis. Increasingly, cases are seen without other clinical evidence of SLE, and the antinuclear antibody may be negative. Indeed, over 10% of myocardial infarctions in young men and 10% of recurrent abortions are said to be a consequence of this autoimmune disease [7, 9]. Recognition is important because of the high risk of multiple thrombotic events. The level of antiphospholipid antibody may fluctuate, so a normal test should be repeated later if the index of suspicion is high. Low levels are often best ignored, particularly as some assays have an unduly low normal range (standardisation is underway).

Treatment is difficult. Venous thrombosis and pulmonary embolism can be controlled by anticoagulation, but there is a high rate of recurrence if anticoagulation is discontinued. Prednisolone at a daily dose of about 40 mg together with a small dose of aspirin has led to successful pregnancy in women with many previous abortions. In some cases treatment may have to begin before conception, but may reduce fertility; management of such pregnancies is currently the subject of clinical trial. Corticosteroid therapy, immunosuppression, control of blood pressure, anticoagula-
tion and antiplatelet agents all have a role in the prevention of arterial thrombosis. Steroids are effective (in that withdrawal of steroid therapy has been known to lead on to a stroke), but the dose and the risk/benefit ratio of long-term treatment are still unknown. Low-dose aspirin therapy may be a wise precaution in patients with high anticardiolipin levels even when they are well.

Antibodies to soluble cellular antigens

Many laboratories are now testing for antibodies to 'extractable nuclear antigens' (ENA) present in a simple saline extract of cells [2, 10–12]. (Some of these antigens are cytoplasmic, so the term ENA is not strictly accurate.) This set of antibodies is detected by the formation of precipitin lines between drops of serum and cell extract placed in wells cut in a gel (using diffusion or electrophoresis). Reference sera are used to seek lines of identity, and in this way a dozen or more different antibodies can be recognised. With purification of the antigens, quantitative assays are now under way, in some cases revealing still closer disease associations and fluctuations in the antibody levels [13]. The arcane nomenclature (Ro, La, Sm, RNP and so on) is based on patients' names, disease associations and crude biochemical analysis, and for some there are synonyms (Ro/SSA, La/SSB), but at least such codes may be easier to recall than the names of the cellular enzymes and particles now known to be involved.

In clinical practice these antibodies are of growing importance for several reasons: they are much more closely associated with disease than the ordinary ANA test; some are positive when the ordinary ANA is negative; and most show remarkable clinical associations with particular syndromes within the connective tissue diseases. Some of these are termed 'disease subsets' (like the thrombotic syndrome in SLE) and others 'overlap syndromes', the best known being mixed connective tissue disease (MCTD). Overlap syndromes vary in severity from patient to patient, but usually there is some combination of Raynaud's phenomenon, myositis, arthritis, scleroderma, sicca syndrome and pulmonary fibrosis. In the early stages any distinction between them may seem academic, but evidence is growing for major differences in outcome, ranging from full recovery to pulmonary fibrosis, pulmonary hypertension, scleroderma or central nervous system disease. Long-term follow-up will better define these overlap syndromes, but already the identification of a particular autoantibody can be used as a piece in the jigsaw of clinical features and laboratory findings that leads to diagnosis and to an informed approach to follow-up. The more important precipitating antibodies are summarised in Tables 1 and 2, and their relationships with particular disease subsets and overlap syndromes are discussed below.

Anti-Ro, SLE and sicca syndrome

Anti-Ro is a relatively common antibody, found in 75% of patients with primary Sjögren's syndrome and up to

| Table 1. Antibody disease associations in the connective tissue diseases. |
|-----------------|--------|-----------------|--------|--------|--------|--------|
|                | SLE    | MCTD | Primary Sjögren's syndrome | Myositis | PSS | RA | PBC | CAH | Other diseases |
| Ro              | 24     | 17   | 75                           | 8       | 4   | 3   | 6   | 4   | <1 |
| La              | 9      | 3    | 42                           | 1       | 1   | -   | 1   | -   | -  |
| Sm              | 4–30   | 3    | -                            | -       | -   | -   | -   | -   | -  |
| RNP             | 23     | 100  | 4                            | 14      | 3   | -   | -   | -   | -  |
| Jo-1            | <1     | 3    | -                            | 25      | 1   | -   | -   | -   | -  |
| PL-7            | -      | -    | -                            | 5       | -   | -   | -   | -   | -  |
| PL-12           | -      | -    | -                            | 3       | -   | -   | -   | -   | -  |
| PM-Scl          | -      | -    | -                            | 11      | 3   | -   | -   | -   | -  |
| Ku              | 6      | -    | -                            | 3       | -   | -   | -   | -   | -  |
| Scl-70          | -      | -    | -                            | 20      | -   | -   | -   | -   | -  |
| Centromereb     | 2      | -    | -                            | 29      | -   | 8   | -   | -   | <1 |
| Multiple nuclear dotsb | 2 | -    | -                            | -       | -   | -   | -   | -   | -  |
| XR              | -      | -    | -                            | -       | -   | -   | -   | -   | -  |
| SL/Kib         | 6      | 3    | 2                            | -       | -   | 3   | 1   | -   | -  |
| Ribosomal       | 3      | -    | 2                            | -       | -   | -   | -   | -   | -  |
| PCNA            | 3      | -    | -                            | 1       | -   | -   | -   | -   | -  |
| PL-4           | 2      | -    | -                            | -       | -   | -   | -   | -   | -  |
| XH             | -      | -    | -                            | -       | -   | -   | -   | -   | -  |

Adapted from Bernstein et al. [2]. Over 1200 sera were tested.

SLE = systemic lupus erythematosus; MCTD = mixed connective tissue disease; PSS = progressive systemic sclerosis; RA = rheumatoid arthritis; PBC = primary biliary cirrhosis; CAH = chronic active hepatitis.

*Sm in 4% of white patients and 30% of black and Chinese, 7% overall.

*Detected by immunofluorescence.
Anti-La and sicca syndrome

Antibody to La nearly always occurs in association with anti-Ro but is less common, being found in just under half the cases of primary sicca (Sjögren's) syndrome and 10% of SLE. In SLE it often indicates a milder course, with rash but no renal disease, going on to sicca syndrome later [16]. Lymphocytic infiltration leading to dryness of exocrine glands, most obvious in the lacrimal and salivary glands, is an important sign of systemic autoimmune disease, whether rheumatoid arthritis, chronic active hepatitis, primary biliary cirrhosis or a connective tissue disease. Anti-Ro and anti-La usually indicate a sicca syndrome that is 'primary' or secondary to SLE. Arthritis is often a feature, with rheumatoid factor strongly positive, yet this differs from rheumatoid arthritis and is rarely destructive or crippling. (As a simple diagnostic point, in a patient with seropositive arthritis and raised ESR, the C-reactive protein is elevated in RA but usually normal in SLE and Sjögren's syndrome.) Sometimes the sicca features are still sub-clinical when the disease presents, but a high IgG level may be a clue and should stimulate a request for antibodies to Ro and La. Our current studies show that high IgG1 subclass is a strong indicator of Sjögren's syndrome in evolution [17].

Congenital heart block

Antibodies to Ro and La are associated with congenital heart block. It was known for some years that a third of mothers delivering a child with congenital atrio-ventricular block have a connective tissue disease, usually SLE or primary Sjögren's syndrome. It is now clear that well over two-thirds of the mothers carry anti-Ro and often also anti-La; these antibodies cross the placenta [18]. Some babies exposed in this way are born with heart block, and a few even have transient rash and hepatosplenomegaly. It appears that antibodies to La and to just one of the three Ro antigens are most often involved [19], but it must be emphasised that only a minority of mothers with either antibody produce babies with heart block. Ro and La are expressed in fetal heart, but it may take an insult such as occult virus infection to expose these antigens on the surface of cardiac cell membranes.

Anti-Sm and SLE

Sm antibody is almost only found in SLE, and like anti-DNA antibody it is now an American classification criterion for the disease. However, there is racial variation in its frequency: whilst common in black and Chinese patients it is rarely encountered in white patients with lupus [2].

Anti-RNP and mixed connective tissue disease

Anti-RNP antibody is found in a quarter of patients with SLE and somewhat less often in systemic sclerosis, primary sicca syndrome and polymyositis. Particularly high titres, with bright speckled ANA staining, are found in a group of patients who lack anti-DNA antibodies but have certain clinical similarities; for these cases the term 'mixed connective tissue disease' has been coined [20]. Invariably these patients have severe Raynaud’s syndrome and swollen fingers, usually together with some, or a few, of the features of SLE,
scleroderma, sicca syndrome, myositis and rheumatoid arthritis. Renal disease is much less common than in SLE, and follow-up suggests that the inflammatory features eventually settle down (sometimes with the antibody disappearing), leading to recovery or more often leaving an end stage with features of scleroderma. It must be admitted that some rheumatologists MCTD is still a controversial concept [21] since, even in typical SLE, anti-RNP antibody is associated with Raynaud’s phenomenon and an increased frequency of myositis, while other patients with the antibody have Raynaud’s syndrome only. Certainly it is not the only overlap syndrome, and its severity can vary. At one time it was taught that MCTD is a benign condition particularly responsive to corticosteroid therapy. In fact, the 10-year mortality, 5–10%, is a little worse than in SLE, and injudicious use of steroids adds infection, osteoporosis and other cushingoid side-effects to the later fibrotic illness. Myositis, depression, psychosis, pleurisy and other inflammatory involvement may require steroid therapy, but the development of interstitial lung disease, pulmonary hypertension and oesophageal dysmotility is not prevented. As in systemic sclerosis, a histamine-H2 receptor antagonist will diminish reflux oesophagitis and the risk of stricture. There is as yet no evidence that calcium-antagonist vasodilators can prevent the development of pulmonary hypertension, but they may be of limited value for Raynaud’s phenomenon. Of theoretical interest, the immunoglobulin level is often twice normal, largely anti-RNP, and fluctuates with the degree of inflammation; it is tempting to speculate that this plays some role in the underlying microvascular disease and spasm. Patients vary in their individual responses to the proteins within the Sm and RNP particles, but new enzyme-linked assays for antibodies to the individual polypeptides give little extra information of clinical value.

Jo-1, PL-7, PL-12, PM-Scl and Ku in myositis

An overlap syndrome somewhat similar to MCTD but with a different pace and presentation has been recognised through studies of Jo-1 antibody [22, 23]. The presentation is usually with myositis or pulmonary fibrosis; 80% of cases have both, and either involvement can be aggressive. Joint pains and Raynaud’s phenomenon usually enter the picture; sicca syndrome and sometimes sclerodactyly develop later, and occasionally there is full-blown lupus or systemic sclerosis with additional autoantibodies detectable. Anti-Jo-1 antibody is found in 25% of cases of myositis in rheumatological practice, but it is not found in childhood myositis and seems to be rare in the myositis associated with malignancy. Two uncommon antibodies, PL-7 and PL-12, are associated with the same syndrome and directed at antigens closely related to Jo-1 in the cell; all are enzymes that charge tRNAs with their respective amino acids [24-26]. Further antibodies, unrelated to the Jo-1 specificity, are anti-PM-Scl and anti-Ku; both seem to be associated with a myositis–scleroderma overlap, in which the emphasis moves from early myositis to later acrosclerosis and calcinosis, often again with pulmonary fibrosis. These antibodies are closely related to myositis syndromes, but anti-Ku also occurs in SLE, at least in our English patients. Follow-up will show just how far these overlap syndromes differ one from another, but already the detection of anti-Jo-1 and the other antibodies is a warning of chronic multisystem disease with the focus on muscle and lung.

Anti-Scl-70 in scleroderma

Anti-Scl-70 antibody is specific for progressive systemic sclerosis and detectable in over 20% of cases [27]. These patients have diffuse skin involvement and an increased risk of pulmonary fibrosis [28, 29]. Contrast this with anti-centromere ANA and the more indolent CREST syndrome, where severe pulmonary hypertension may eventually develop. In a patient with Raynaud’s syndrome, the presence of either antibody suggests progression to scleroderma.

Further autoantibodies in SLE

SLE is not a blizzard of innumerable autoantibodies, but a few more precipitins do occur, with frequencies of 3–10%. These include the Ki(SL), PCNA, Ku, ribosomal and PL-4 systems [2]. Antibodies to PCNA and PL-4 are rare but specific for SLE, whereas the others are less restricted. In SLE, acute neuropsychiatric lupus is associated with antibody to a ribosomal phosphoprotein. There is more non-infective fever with anti-SL and more renal disease with PL-4.

Anti-XR and chronic active hepatitis

Not only the connective tissue diseases have their ‘ENAs’. In autoimmune chronic active hepatitis there is in a quarter of cases a precipitating antibody called anti-XR [30]. Anti-XR and two other precipitins, XR-2 and XH, are also found in primary biliary cirrhosis (PBC), but there the frequencies are below 10%. More common in PBC (as well, of course, as the almost ubiquitous antimitochondrial antibody) are two ANA specificities detected by immunofluorescence in tissue culture cells: anti-centromere antibody and a rather similar speckled staining called ‘multiple nuclear dots’ [30]. One-fifth of patients with PBC develop features of CREST syndrome, and half of these have anti-centromere antibody. Multiple nuclear dots antibody, on the other hand, is found mainly in PBC with sicca syndrome.

Antibodies to neutrophil cytoplasm in vasculitis

Once invariably fatal, Wegener’s granulomatosis is now curable with steroids and cytotoxic therapy. Early treatment is vital, yet diagnosis is often delayed because the high neutrophil leukocyte count suggests infection or because a tissue diagnosis is insisted on. The discovery
of autoantibodies to neutrophil cytoplasm—sometimes to myeloperoxidase—in Wegener’s granulomatosis, microscopic polyarteritis and (of IgA class) in Henoch–Schönlein purpura has simplified diagnosis and the monitoring of disease activity [31].

Antibodies to cellular stress proteins

Cells respond to a variety of stresses such as hyperthermia by upregulating the synthesis of several ‘heat-shock’ proteins. Highly conserved from bacteria to man, the response is protective, for instance stabilising immature proteins (cellular society still has its chaperones). Antibodies to human hsp70 and hsp90 are found in SLE and to bacterial hsp65 in RA and ankylosing spondylitis [32]. T-lymphocytes from arthritic joints react with a mycobacterial 65 kD heat-shock protein (hsp65) and T-cells sensitised to hsp65 can experimentally transmit, suppress or immunise against adjuvant arthritis in rats. Pretreatment with hsp65 itself can prevent arthritis in several experimental models. This is hot news but, whether profound or just topical, time will tell.

HLA associations with autoantibodies and their syndromes

There are well known associations between the major histocompatibility complex and rheumatic disease, such as HLA-B27 with ankylosing spondylitis, HLA-DR4 with rheumatoid arthritis, and in SLE and Sjögren’s syndrome the A1, B8, DR3 haplotype together with null alleles of the fourth component of complement, C4A. Associations have also been recorded between some autoantibodies and HLA: anti-La with the DRW52 form of DR3, anti-Jo-1 with DR3, anti-RNP with DR4 and anti-centromere with DR1 and DR5. The La and Jo-1 associations are particularly close and immune response genes have been invoked. However, the mechanism remains unclear, even as to whether HLA is tied more to the antibody or primarily to the associated clinical syndrome.

Autoantigens in the life of the cell

In parallel with their clinical evaluation, autoantibodies are proving powerful tools for molecular biologists. There are some 100,000 intracellular macromolecules, yet well under 50 are autoantigens. Of these a few are nucleic acids (notably DNA itself) while most are proteins. Often the antigen is part of a macromolecular complex with other protein and nucleic acid components [2]. In such a complex, one or another component can be antigenic in different patients [33], showing that the whole particle becomes involved in the antibody response. It is hard to see how this could be a result of random polyclonal B-cell activation or tissue damage, but a virus infecting the cell might bind to a particular cellular component, so rendering it immunogenic [24], or a fortuitous cross-reaction with a foreign immunogen (the rheumatic fever model of molecular mimicry) might initiate the loss of tolerance to self. As shown in Table 3, most of the antigens have vital roles in the replication of DNA and the synthesis of proteins. For instance, histones are structural proteins around which the DNA is coiled and supercoiled; the Scl-70 antigen is an enzyme, topoisomerase I, that unwinds the supercoils so that the DNA can be replicated or transcribed [34], and the proliferating cell nuclear antigen is involved in DNA synthesis [35]. One of the nucleolar antigens is an RNA polymerase, and Sm and RNP are small ribonucleoprotein particles that cut out redundant sequences from RNA transcripts to make messenger RNA [36]. The ‘myositis antigens’ Jo-1, PL-7 and PL-12 are three of the enzymes that pair up amino acids with their appropriate transfer RNAs ready for protein synthesis (translation) at the ribosomes [24–26].

**Table 3. Intracellular antigens in the life of the cell.**

| Antigen   | Identity                | Function                                                                 |
|-----------|-------------------------|--------------------------------------------------------------------------|
| Histones  | Structural proteins     | Packaging of DNA                                                         |
| Scl-70    | Topoisomerase I         | DNA supercoiling                                                         |
| PCNA      | 35K protein             | Auxiliary factor for DNA polymerase δ                                    |
| Ku        | 60K, 80K proteins       | DNA binding                                                              |
| Nucleolar | RNA polymerase I        | Transcription of rRNA                                                     |
| spckled   | (U1)ribonucleoprotein   | RNA processing                                                           |
| RNP       | particle (68K, A, C proteins) |                                                                 |
| Sm        | Several (U)ribonucleoprotein particles (B', D proteins) |                                                   |
| Jo-1, PL-7, PL-12 | Aminoacyl-tRNA synthetases (his, thr, ala) |                                                                 |
| Ribosomal RNP | Ribosomal P proteins and rRNA | Protein synthesis                                                         |
| La        | 46K protein             | Termination of RNA polymerase III transcription                          |
| Ro        | 52K, 54K, 60K proteins  | RNA transport or translation control                                       |

New insights

From studies of the autoantigenic targets, several generalisations can be drawn (Table 4). First, the autoantibodies react with elements of the replicative and protein synthetic apparatus of the cell. Second, the functional enzymatic activity of the antigens can be inhibited *in vitro*—something not achieved by many experimental antibodies. Third, where antibodies occur together in patients, such as anti-Sm with anti-RNP and anti-La with anti-Ro, this is mirrored in an association of the antigens within the cell: the Sm and RNP polypeptides are components of the (U1)RNP complex and likewise the Ro and La antigens are associated at least transiently. A further observation, based
on idiotype studies and sequencing of monoclonals, is that anti-DNA antibodies are derived from the same germ line genes as several antibacterial antibodies [37]. The cDNAs have been cloned for several antigens (Ro, La, major Sm and RNP proteins, Jo-1, PCNA, Ku, Scl-70 and ribosomal P protein), and recombinant proteins can be used as the substrate for antibody assays. Mapping of the antigenic epitopes reveals a complex picture with differences from patient to patient, and analysis of antibodies by electrofocusing, using pure antigen to label the immunoglobulin bands, shows the response is polyclonal. All this suggests that autoantibodies are the result of an antigen-driven response.

**Origin of autoantibodies: clues to aetiology**

Autoantibodies are unlikely as a rule to enter cells and inhibit the function of their antigens directly. However, certain stresses such as ultraviolet radiation or virus infection can lead to the expression of antigens on the cell surface where antibody can bind. Cell death, of course, releases antigens to the circulation whence immune complexes may form. Thus, the damaging effects of an autoantibody (ill-defined as these still are) may account for some of the clinical associations (eg of anti-Ro with congenital heart block and the rash of subacute cutaneous LE). However, this still does not account for the association of some autoantibodies with each other, or explain clinical associations where the pathogenesis is thought to be mediated by lymphocytes directly (eg polymyositis).

The idea has arisen that autoantibodies are clues to aetiology, reporters of past events. Whether the autoantibody is pathogenetic or an innocent bystander, it may have arisen as a result of the same virus infection that triggered the disease. Three hypotheses have been proposed for this: through molecular mimicry [38], through a molecular complex of viral and host cell components overcoming immunological tolerance [24], and through an anti-idiotype response to antiviral antibodies (where the antiviral antibody's combining site mimics a receptor for the viral ligand) [39]. Evidence can be adduced for all these hypotheses. For molecular mimicry there is a sequence homology between an epitope on the 68 kD protein of (U1)RNP and the p30 group antigen of murine leukaemia virus [38], together with a second homology between an Scl-70 epitope and an adjoining sequence on the same p30gag. For the virus-host interaction model, there are known interactions of small RNAs from adenovirus and Epstein–Barr virus with La antigen, and of meningoovirus RNA with Jo-1. For the idiotype model there is, for instance, the anti-anti-mycoplasma antibody with anti-I cold agglutinin activity, and also the coexistence of antibodies to tRNA\(^*\)ua and alanyl-tRNA synthetase in myositis [26].

Currently, there are fruitful studies underway looking at autoimmune responses in transgenic mice [40] and for retroviruses in humans with connective tissue diseases.

**References**

1. Grabar, P. (1983) Autoantibodies and the physiological role of immunoglobulins. *Immunology Today*, 4, 385–9.
2. Bernstein, R. M., Bunn, C. C., Hughes, G. R. V. *et al.* (1984) Cellular protein and RNA antigens in autoimmune disease. *Molecular Biology and Medicine*, 2, 105–20.
3. Moroi, Y., Peebles, C., Fritzier, M. *et al.* (1989) Autoantibody to centromere (kinetochore) in serologically normal sera. *Proceedings of the National Academy of Sciences of the USA*, 77, 1627–31.
4. Hargraves, M. M., Richmond, H. and Morton, R. (1948) Presentation of two bone marrow elements: the ‘Tart’ cell and the ‘L.E.’ cell. *Proceedings of the Mayo Clinic*, 23, 25–8.
5. Hughes, G. R. V. (1979) The connective tissue diseases, 2nd edn. Oxford: Blackwell Scientific Publications.
6. Harris, E. N., Gharavi, A. E., Boey, L. L. *et al.* (1983) Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in SLE. *Lancet*, ii, 1211–4.
7. Byron, M. (1989) Anticardiolipin antibody in pregnancy—to treat or not? *Current Medical Literature*, 8, 67–71.
8. Harris, E. N., Gharavi, A. E. and Hughes, G. R. V. (1985) Antiphospholipid antibodies. *Clinics in Rheumatic Diseases*, 11, 591–609.
9. Hamsten, A., Norberg, R., Bjorkholm, M. *et al.* (1986) Antibodies to cardiolipin in young survivors of myocardial infarction: an association with recurrent cardiovascular events. *Lancet*, ii, 115–6.
10. Anderson, J. R., Gray, K. G., Beck, J. S. and Kinnear, W. P. (1961) Precipitating autoantibodies in Sjögren’s disease. *Lancet*, ii, 456–60.
11. Tan, E. M. (1982) Autoantibodies to nuclear antigens: their immunobiology and medicine. *Advances in Immunology*, 33, 167–240.
12. Reichlin, M. (1981) Current perspectives on serological reactions in SLE patients. *Clinical and Experimental Immunology*, 44, 1–10.
13. Venables, P. J. W., Charles, P. J., Buchanan, R. R. C. *et al.* (1983) Quantitation and detection of isotypes of anti-SS-B antibodies by ELISA and Farr assays using affinity purified antigens: an approach to the investigation of Sjögren’s syndrome and systemic lupus erythematosus. *Arthritis and Rheumatism*, 26, 146–55.
14. Alexander, E. A. and Provost, T. T. (1981) Ro (SSA) and La (SSB) antibodies. *Springer Seminars in Immunopathology*, 4, 253–73.
15. Maddison, P. J. (1982) ANA-negative SLE. *Clinics in Rheumatic Diseases*, 8, 105–19.
16. Wasicck, C. A. and Reichlin, M. (1982) Clinical and serological differences between systemic lupus erythematosus patients with antibodies to Ro versus patients with antibodies to Ro and La. *Journal of Clinical Investigation*, 69, 835–45.
17. Hay, E. M., Freemont, A. J., Kay, R. A. *et al.* (1989) Selective polyclonal elevation of immunoglobulin G1 subclass: a link with Sjögren’s syndrome. *Annals of the Rheumatic Diseases*, 48, in press.
18. Scott, J. S., Maddison, P. J., Taylor, P. V. *et al.* (1983) Connective-
tissue disease, autoantibodies to ribonucleoprotein, and congenital heart block. New England Journal of Medicine, 309, 209–12.

19. Buyon, J. P., Ben-Chetrit, E., Karp, S., et al. (1989) Association of congenital complete heart block with maternal antibody response to the novel 52 kD SSA/Ro (abstract). Arthritis and Rheumatism, 32, S104.

20. Sharp, G. C., Irvin, W. S., Tan, E. M. et al. (1972) Mixed connective tissue disease: an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). American Journal of Medicine, 52, 148–59.

21. Alarcon-Segovia, D. (1981) Mixed connective tissue disease: a decade of growing pains. Journal of Rheumatology, 8, 555–40.

22. Yoshida, S., Akizuki, M., Mimori, T. et al. (1983) The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases: a marker for a subset of polymyositis with interstitial pulmonary fibrosis. Arthritis and Rheumatism, 26, 604–11.

23. Bernstein, R. M., Morgan, S. H., Chapman, J. et al. (1984) Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. British Medical Journal, 289, 151–2.

24. Mathews, M. B. and Bernstein, R. M. (1985) Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. Nature, 314, 177–9.

25. Mathews, M. B., Reichlin, M., Hughes, G. R. V. and Bernstein, R. M. (1984) Anti-threonyl-tRNA synthetase, a second myositis-related autoantibody. Journal of Experimental Medicine, 160, 420–34.

26. Bunn, C. C., Bernstein, R. M. and Mathews, M. B. (1986) Autoantibodies to alanyl-tRNA synthetase and tRNAα coexist and are associated with myositis. Journal of Experimental Medicine, 163, 1281–91.

27. Douvas, A. S., Achten, M. and Tan, E. M. (1979) Identification of a nuclear protein (Scl-70) as a unique target of human anti-nuclear antibodies in scleroderma. Journal of Biological Chemistry, 254, 10314–22.

28. Bernstein, R. M., Steigerwald, J. C. and Tan, E. M. (1982) Association of antinuclear and antineculeolar antibodies in progressive systemic sclerosis. Clinical and Experimental Immunology, 48, 43–51.

29. Catoggio, L. J., Bernstein, R. M., Black, C. M. et al. (1983) Serological markers in progressive systemic sclerosis: clinical correlations. Annals of the Rheumatic Diseases, 42, 23–7.

30. Bernstein, R. M., Neuberger, J. M., Bunn, C. C. et al. (1984) Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. Clinical and Experimental Immunology, 55, 3–60.

31. Falk, R. J. and Jennette, J. C. (1988) Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotising and crescentic glomerulonephritis. New England Journal of Medicine, 318, 1651–7.

32. Bernstein, R. M. (1989) Heat shock proteins and arthritis. British Journal of Rheumatology, 28, 360–71.

33. Habets, W. J., Moet, M. M., van de Pas, J. and van Venrooij, W. J. (1985) Characterisation of nuclear and cytoplasmic autoimmune antigens. In Proteins of the biological fluids (ed. J. Peeters) pp 199–204. Oxford: Pergamon.

34. Shero, J. H., Bordwell, B., Rothfield, N. F. and Earnshaw, W. C. (1986) High titres of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science, 231, 737–40.

35. Mathews, M. B., Bernstein, R. M., Franz, R. and Garrels, J. I. (1984) The identity of the ‘proliferating cell nuclear antigen’ and ‘cyclin’. Nature, 309, 374–6.

36. Lerner, M. R., Boyle, J. A., Mount, S. M. et al. (1980) Are snRNPs involved in splicing? Nature, 283, 220–4.

37. Sela, O., Arutyunyan, A., Isenberg, A. et al. (1987) A common anti-DNA idiotype in sera of patients with active pulmonary tuberculosis. Arthritis and Rheumatism, 30, 50–6.

38. Query, C. C. and Keene, J. D. (1987) A human autoimmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30 gag antigen. Cell, 51, 211–20.

39. Plotz, P. H. (1983) Autoantibodies are anti-idiotypic antibodies to viral antibodies. Lancet, ii, 824–6.

40. Green, J. E., Hinrichs, S. H., Vogel, J. and Jay, G. (1989) Exocrinopathy resembling Sjogren's syndrome in HTLV-1 tax transgenic mice. Nature, 341, 72–4.