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*Review article*

# The value of specific ANA determination in rheumatology

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## *Introduction*

Since the discovery of the lupus erythematosus cell in 1948 by Hargraves et al. (66) and the subsequent identification of antibodies capable of opsonizing whole cell nuclei, thereby promoting their ingestion by neutrophilic granulocytes (73), a vast array of antinuclear antibodies (ANA) have been found to exist in systemic inflammatory rheumatic diseases. It is the intention of this communication to survey some of the advances made in ANA serology with regard to recognition of the biochemical nature of many nuclear antigens, the clinical value of ANA determination in diagnosing particular rheumatic diseases and potential value of monitoring certain diseases by quantitative ANA determinations during follow-up. Finally, some aspects which seem to link ANA with possible etiologic and pathogenetic mechanisms will be touched upon. Technical details of ANA determination have largely been left out, but methodologic key references are alluded to in the text. Table 1 lists the disease associations, diagnostic specificity and nosologic sensitivity of various ANA, and molecular nature of the corresponding antigens.

## *Indirect immunofluorescence screening for ANA*

Indirect immunofluorescence technique (54, 72) has been and still is the preferred method by which sera are screened for presence of ANA. Earlier studies mostly used tissue cryostate sections or nucleated blood cells as substrates for reaction (49). More recently, various types of cultured human cell lines have come into use, the advantages being a constant availability of human cells of a well-characterized cellular derivation, nuclear morphology and antigenic composition obtainable on a large scale (65). Also, the presence of cells in various stages of proliferation and division is of importance, since some ANA are best recognized on such cells. A particular cell line may contain a number of desired nuclear antigens and still lack some important ones (158). Therefore, a reasonable compromise usually has to be reached with regard to completeness of desired antigen build-up, richness in nuclear details and variety in subcellular distribution of antigens during mitosis.

Indirect immunofluorescence technique has a number of advantages as a screening method for ANA: 1) it discloses the localisation of a given nuclear antigen against which the ANA is directed, and the staining pattern in resting and mitotic cells may thereby give an indication as to the fine specifity of the ANA (76, 119), 2) it possesses a reasonably high serologic sensitivity (49, 65), 3) it can categorize the ANA as belonging to one, two or more Ig classes or subclasses by use of appropriate anti-Ig conjugates as tracer antibodies (181), 4) it can disclose the complement-fixing properties of the ANA if sufficient complement is added to the system, and the complement activation is traced by specific anti-complement conjugates (177), 5) it functions in principle as a solid-phase immunoassay provided that the nuclear antigen under study can be insolubilized properly by

Table 1

Antinuclear antibodies associated with systemic inflammatory rheumatic diseases

| Antibody | Major disease association | Diagn. spec. | Nosol. sensit. % | Molecular characteristics |
|---|---|---|---|---|
| GS-ANA | RA | moderate | 50-80 | |
| Anti-RANA | RA | moderate | 80-90 | 80 kD protein |
| Anti-dsDNA | SLE | high | 50-60 | |
| Anti-RNA species | multiple | low | | 4-6S RNA, tRNA, rRNA = $PL_{-3}$. |
| Anti-histones | drug-induced LE | low | 90-95 | H1, H2B, H3. |
| Anti-DNP | SLE | moderate | 40-60 | |
| Anti-nRNP | MCTD | low | 95-100 | U1 RNP, 70,31,19 kD mRNA splicing enzyme |
| Anti-Sm | SLE | high | 15-30 | U1,2,4,5,6 RNP, 25, 13 kD protein |
| Anti-SS-A | 1° Sjögren's syndrome | low | 80-100 | 55 kD protein assoc. with Y1 RNA |
| Anti-SS-B | 1° Sjögren's syndrome | low | 70-95 | 50,43 kD protein assoc. with snRNAs |
| Anti-PCNA | SLE | moderate | 5 | 36 kD protein, cyclin |
| Anti-Scl-70 | PSS | high | 20-60 | 70,100 kD protein, DNA topoisomerase I |
| Anti-Scl-A | PSS | high | 30-40 | RNA polymerase I |
| Anti-Scl-B | PSS-PM overlap | moderate | 10-15 | 26-36 kD proteins |
| Anti-Scl-C | PSS | high | | U3 RNP,34 kD protein |
| Anti-centromere | CREST syndrome | high | 60-90 | 140,80,19 kD proteins |
| Anti-PM-1 | PM | high | 60-70 | Several proteins |
| Anti-Jo-1 | PM | high | 25-30 | 50 kD, histidyl-tRNA synthetase |
| Anti-PL-7 | PM | high | ? | 80 kD, threonyl-tRNA synthetase |
| Anti-PL-12 | PM | ? | 1 | alanine tRNA synthetase |
| Anti-Mi-1 | DM | moderate | 10 | 150 kD protein |
| Anti-Mi-2 | DM | high | 20 | 53,61 kD proteins |
| Anti-Ku | PM-PSS overlap | moderate | 50-60 | 300 kD protein |
| Anti-SL | SLE-sicca | moderate | 5-10 | 32 kD protein |
| Anti-PL-4 | SLE | high | 20 | 150 kD protein |
| Anti-Ma | SLE | high | 20 | acidic protein |
| Anti-PL-8 | SLE | high | 1 | 96 kD phosphoprotein |
| Anti-PL-9 | SLE | high | 1 | 65,67,86 kD prot. |
| Anti-Su | SLE | high | 4 | 150 kD protein |
| Anti-protein kinase NII | SLE | moderate | ? | 140 kD, protein, kinase NII |
| Anti-nuclear dots | several | low | | 43 kD protein |
| Anti-nuclear envelope | linear scleroderma | | | 60,70 kD protein, lamins. |
| Anti-mitotic spindle appar. | several | low | | |

U RNA/RNP = small nuclear RNA/RNP.
Y RNA/RNP = cytoplasmic RNA/RNP.

fixatives which leave antigenic reactivity intact. In this way test sample ANA content and complement-fixing abilities may be quantitated by titration (128). However, it is important to mention that ANA may be directed towards nuclear antigens which are highly soluble in physiologic buffers and which cannot be insolubilized by fixatives applicable to immunohistochemical methodology. In these cases very diligent handling of cell preparations is indispensable upon fixative treatment since the accessibility promoting activity of the fixative simultaneously increases the risk of antigen extraction and hence disturbance of the ANA staining pattern.

### *Nuclear staining patterns*

Several studies have indicated that the human epidermal tissue culture cell line, HEp-2 cells,

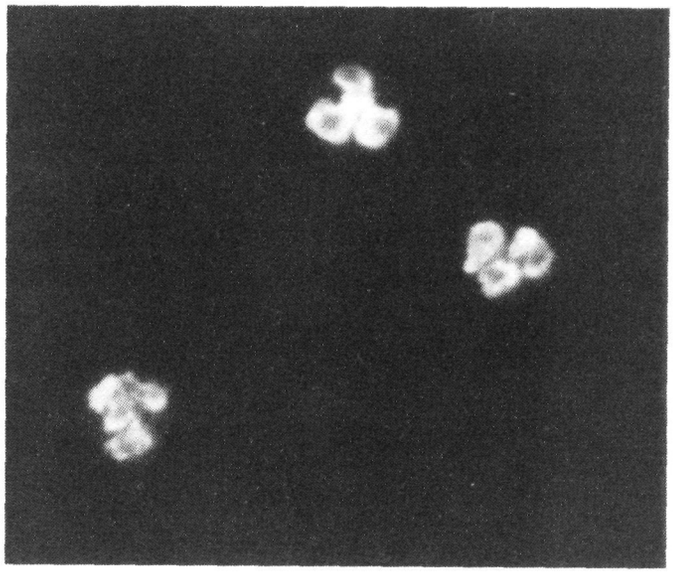


Fig. 1. Granulocyte-specific ANA (human leucocytes).

combines easil differentiable cellular details and a high percentage of mitotic cells with presence of most of the important nuclear antigens desired in a substrate for ANA screening (76). With these cells as substrate a number of characteristic staining patterns can be delineated (Figs. 2, 3, 5–12). A few of these patterns are related to ANA directed to single morphologically identifiable nuclear antigens i.e. anti-centromere antibodies (ACA), anti-proliferating cell nuclear antigen (anti-PCNA) and anti-mitotic spindle apparatus (anti-MSA) (Figs. 7, 10, 12). Other patterns of nuclear fluorescence can merely help the serologist plan further studies of ANA specificity as shown by other serologic methods such as precipitation, passive hemagglutination, radio-immunoassay or enzyme-linked immunosorbent assay (ELISA), each of which will reveal only one ANA specificity.

Two specific ANA systems will be mentioned first, since special cellular substrates are needed for their identification.

*Granulocyte-specific antinuclear antibodies (GS-ANA)*

Antibodies specifically directed to nuclear antigens of mature neutrophilic granulocytes and monocytes were originally described in a patient with Felty's syndrome by use of indirect immunofluorescence technique on human donor leucocytes (48, Fig. 1). Further studies showed that these so-called granulocyte-specific (GS-) ANA are common in patients with rheumatoid arthritis (RA), being found in about (75%) of patients with active disease, and especially in cases exhibiting autoimmune neutropenia (about 90–95%) (180). IgG GS-ANA may also be found in the active phase of drug-induced lupus syndrome (167). Titres are very high in neutropenic cases of RA where the IgG GS-ANA possess pronounced complement fixability (177) and commonly participate in circulating immune complexes (178). GS-ANA may belong to all of the four subclasses of IgG (181). They are more commonly found in RA patients with active synovitis than in randomly selected patients (128, 179). GS-ANA are also frequently present in girls with polyarticular onset juvenile RA in contrast to oligoarticular and acute febrile onset cases (129). They are found in one fourth of patients with ulcerative colitis but not in Crohn's disease (122). Also in rheumatoid joint fluid GS-ANA commonly form immune complexes that are selectively enriched with these antibodies (176). In prospective studies of RA patients it seems as if presence of IgG GS-ANA is related to more aggressive erosive joint disease than presence of organ-nonspecific ANA or absence of ANA (39, 166).

The antibodies most probably are directed to several highly immunogenic DNA-associated non-histone proteins (180).

*Antibodies to rheumatoid arthritis-associated nuclear antigen (RANA)*

In 1976 Alspaugh et al. described the presence of precipitating antibodies to a nuclear antigen which was present in Epstein-Barr virus (EBV)-infected lymphoblastoid cell lines, and these antibodies were named rheumatoid arthritis precipitins (RAP) (12). The antigen (termed RANA) was strictly associated with EBV transformed cells both upon *in vitro* infection of normal human B cells (15), and with cell lines known to harbor the EBV genome. RANA is located in a finely speckled pattern in the nucleus as visualized by anti-RANA antibodies and indirect immunofluorescence technique (12).

RANA is an extremely soluble protein antigen, sensitive to trypsin but not to DNA-ase or RNA-ase, possibly identical to Epstein-Barr nuclear antigen (EBNA), an 80 kD molecular weight protein of EBV-transformed lymphocytes (25, 31, 36). The RANA/EBNA readily associates with DNA, is highly immunogenic and contains repeating antigenic epitopes rich in glycine and alanine (71).

Anti-RANA antibodies are found in nearly all cases of sero-positive RA (31, 121), but also in many sero-negative cases and in normal controls (30, 31, 121). The main difference between sero-positive RA and other patient groups may be the higher titers encountered in the former group (36). The highest titers of anti-RANA are found in RA patients during active phases of the disease (14). The increased levels of antibodies to RANA/EBNA as well as to EBV early antigen (158) may reflect a specific functional defect of regulator T lymphocytes towards EBV propagation in B lymphocytes (163). This defect can be corrected in RA patient lymphocyte cultures by the addition of normal T cells. Even if EBV is not regarded as an etiologic agent in RA, the particular abnormal host defense against EBV may have pathophysiologic importance, since EBV-infected B lymphocytes produce large amounts of autoantibodies, especially rheumatoid factors (29). A 62 kD glycine-alanine rich protein showing cross-reactivity with RANA has just been discovered in synovial lining cells from RA patients (51), adding weight to the hypothesis that EBV may play a role in the pathogenesis of RA.

*Antibodies to DNA*

Antibodies to DNA fall into at least three categories of specificity: 1) antibodies reacting only with double-stranded (ds) DNA, possibly recognizing conformational determinants in the groves of the double helix, 2) antibodies reacting both with dsDNA and ssDNA recognizing determinants on the sugar-phosphate backbone and 3) antibodies reacting solely with ssDNA recognizing the bases in ssDNA, mononucleotides and mononucleosides (150). The first type of anti-dsDNA is only rarely found in systemic lupus erythematosus (SLE) patients (158). Studies on monoclonal antibodies showing the specificity indicate, that these antibodies never show cross-reactivity with other nucleic acid derivatives or unrelated antigens (15). The second type of anti-dsDNA is commonly detected in SLE, but is infrequent in other diseases and thus valuable diagnostically (158).

This type of anti-dsDNA shows extensive cross-reactivity with phospholipids such as cardiolipin (80, 146), cell surface membrane components (77), coagulation factors (37), and glucosaminoglycans (47). The common denominators in this array of apparently unrelated antigens may be repetitive phosphodiester groups which are relatively negatively charged and/or similarities in polypeptides common to many proteins.

The third group of anti-DNA directed to DNA bases are very commonly found in SLE, other inflammatory rheumatic and non-rheumatic inflammation (158), possibly fulfilling a role in the physiologic processes aimed at eliminating foreign and self DNA (150). Studies on monoclonal antibodies to DNA indicate that other specificities may exist, and among them antibodies that recognize epitopes common to DNA, RNA and cytoskeletal filaments (150).

Anti-dsDNA studied by indirect immunofluorescence on any nuclear substrate produce a homogeneous and/or peripheral nuclear staining pattern (Fig. 2), which cannot be distinguished morphologically with any certainty from anti-deoxyribonucleoprotein (anti-DNP) and anti-histone antibodies (Fig. 3). Aarden introduced the *Crithidia luciliae* trypanosome as a cellular substrate for the demonstration of anti-dsDNA (2, Fig. 4), since the kinetoplast of the parasite was supposed to contain pure dsDNA. However, recently it has been suggested that histones may be present in this organelle (40). If this is true the kinetoplast immunofluorescence test is less valuable for demonstrating anti-dsDNA. Anti-ssDNA cannot be demonstrated by immunofluorescence on interphase cells, whereas they can produce an irregular filamentous or web-like staining pattern on dividing cells (155). The radio-precipitation technique introduced by Pincus in 1969 and modi-

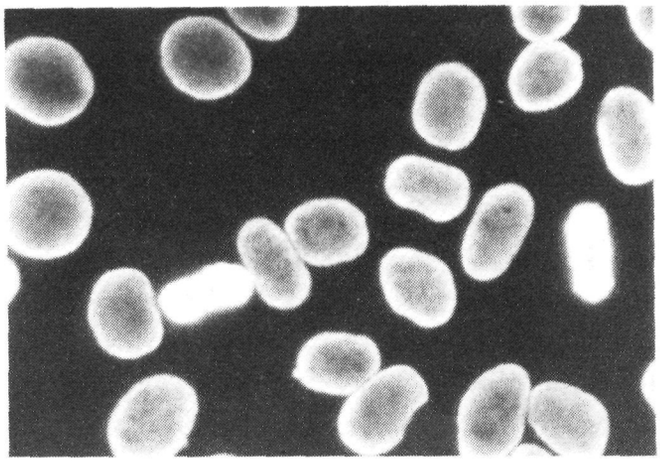


Fig. 2. Peripheral and homogeneous nuclear staining (HEp-2 cells).

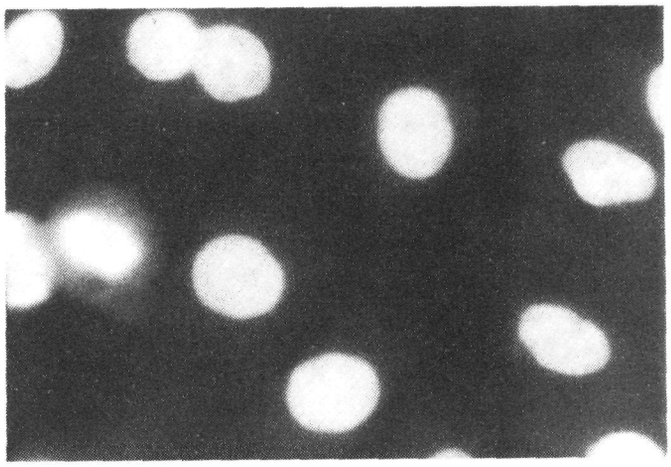


Fig. 3. Homogeneous nuclear staining (HEp-2 cells).

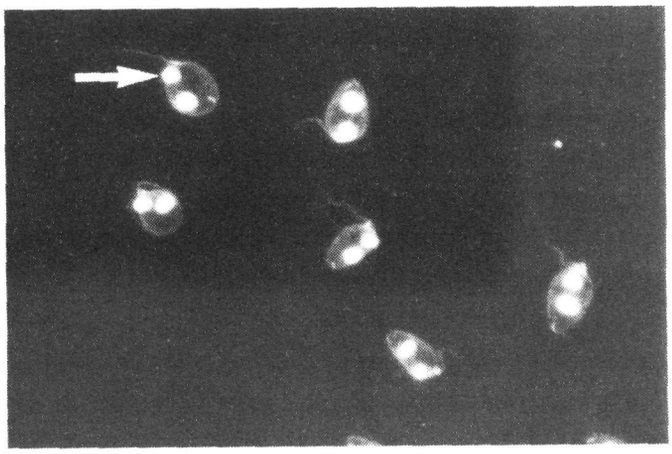


Fig. 4. Nuclear and kinetoplast staining (arrow) by anti-DNA antibodies (*Cririthidia luciliae*).

fications of this method have become the most widely used way of quantitating anti-dsDNA content (130, 158). Radioactively labeled dsDNA is precipitated after binding to anti-dsDNA by 2M ammonium sulphate and the relative amount of labeled antigen in the precipitate taken as a measure of anti-dsDNA content. This method has a number of limitations: 1) it dose not identify the DNA binding components in test samples as being of Ig nature, 2) the assay only measures high affinity anti-dsDNA (147), and 3) the results are very dependent on the double-strandedness and molecular size as well as the purity of the DNA antigen (1). Many ELISA systems have been described for the quantitation of anti-dsDNA to avoid at least some of these drawbacks, but for reasons that are not clear the development of a readily standardized assay has not been accomplished. A more precise quantitation of anti-dsDNA therefore awaits further methodologic advances. The diagnostic specificity of a positive Crithidia kinetoplast test for SLE is clearly lower than that of the Farr test, primarily because it discloses low affinity anti-DNA antibodies which are found in many connective tissue diseases (147).

The nosologic sensitivity of anti-dsDNA for SLE is usually judged to be around 60% when Farr radioprecipitation assays are being used. Sequential studies of these antibodies in SLE patients are recommended for the following reasons: 1) a constant low level of anti-dsDNA indicates inactive disease and a good prognosis, 2) a constant high level may also be a reassuring sign of good prognosis, 3) rapidly increasing levels within a few weeks predict imminent exacerbation of the disease, and 4) the sudden disappearance of anti-dsDNA is seen only during disease exacerbations (153), where considerable concentrations of free circulating DNA may be found (157). The detection of high avidity anti-dsDNA in sera of non-SLE patients is a strong prognosticator for the development of overt SLE, since more than 85% of these individuals ultimately fulfill the criteria for SLE (152).

The mechanisms by which anti-dsDNA may influence the immunopathogenesis of SLE are as yet not well characterized. Though several authors have shown presence of circulating DNA/anti-DNA complexes in SLE (158) and anti-dsDNA have been detected on the glomerular basement membrane in highly enriched concentrations over

serum levels (78), the mechanism(s) of tissue deposition is still unclear. It has been suggested that dsDNA may be strongly bound to collagen type IV through fibronectin and thus in turn may cause deposition of anti-dsDNA in the glomeruli (83). Recent investigations have indicated that a relatively alkaline subpopulation of anti-dsDNA may bind directly to glomerular basement membrane (38), or to negatively charged glycosaminoglycans (44, 47). This binding of anti-DNA may directly cause tissue damage by blocking of anionic sites in the glomerular basement membrane or by complement activation (44). Tissue damage may also be enhanced by the binding of rheumatoid factors to tissue-fixed anti-DNA, thereby increasing immune complex size and complement activation (44). Anti-DNA reacting with surface membrane polypeptides common to many cells, including glomerular and neuronal cells, may have the same effect (77). However, the possibility still exists that soluble intermediate size complement-fixing anti-dsDNA/DNA immune complexes may partially escape the erythrocyte immune complex clearance mechanism and subsequently deposit in certain areas of the vascular bed (161).

A completely different pathophysiologic aspect concerns the possible internalisation of IgG anti-dsDNA into Fcγ-receptor carrying cells such as lymphocytes and monocytes causing functional disturbances of the cells through interaction with nucleic acid metabolism (6), which in turn may explain the impairment of immunoregulatory mechanisms and immune complex clearance through the mononuclear phagocyte system. However, this assumption has not yet been experimentally supported by studies in other laboratories. The fact that anti-ssDNA are uninteresting from a diagnostic viewpoint should not be confused with their role in pathogenic terms. ssDNA has been found complexed to anti-ssDNA in glomeruli of SLE patients indicating their likely pathogenic role in lupus nephritis (79).

*Antibodies to RNA*

Antibodies to RNA have never gained much attention in diagnosing diseases. In up to 50% of patients with progressive systemic sclerosis (PSS) one may find antibodies to nucleoli. Antibodies giving rise to a homogeneous staining pattern of the nucleolus were thought to be directed towards 4–6S RNA molecules (131). More recently, antibodies to heterogeneous nuclear RNA (hnRNA) have been described in SLE (55). Antibodies to 5–6S ribosomal RNA may also be found in SLE (84, 158). Antibodies to antigen Mas, which are found in a few patients with myositis, are directed to a single species of tRNA (21). Antibodies to ribonucleoprotein particles will be dealt with later in this review.

*Antibodies to histones*

Histones are alkaline lysine- and arginine- rich proteins constituting essential parts of the nucleosomes (106). The monomeric nucleosome consists of two of each histone H2A, H2B, H3 and H4, which together form an octameric component around which DNA of a 146-base pair length is wrapped. The individual nucleosomes are connected by link DNA and H1 histone.

Since histones themselves have anti-complementary properties the demonstration of anti-histone antibodies by complement-fixation did not come into wider use (81). The histone extraction and reconstitution immunofluorescence test (56) and later on RIA (140) and ELISA (139) techniques have been used to detect anti-histone activity. About 95% of patients with drug-induced LE syndrome produce anti-histone antibodies (56). However, even one third of asymptomatic patients receiving the same drugs harbor anti-histone antibodies (46). The antibodies were originally assumed to react mainly with the H2A-H2B complex, but later studies using a sensitive fluorometric assay indicate greater heterogeneity of the response (22, 23). A more restricted response to H1 and H2B is seen in 30% of SLE patients, the highest concentrations being found in patients having a history of photosensitivity. Antibodies to histones may be found in about 25% of RA patients (3) especially in cases with vasculitis (23). In RA the antibodies often seem to represent rheumatoid factors cross-reacting with histone. Anti-histone antibodies usually

produce a homogeneous staining pattern in indirect immunofluorescence (Fig. 3).

*Antibodies to deoxyribonucleoprotein (DNP)*

Antibodies responsible for the LE cell phenomenon described by Hargraves in 1948 (66) were later shown to be directed to insoluble DNP (73). Both the DNA and the histone part of the DNP complex seemed to be required for reactivity with these antibodies. Complement-fixing properties mainly ascribed to IgG class anti-DNP were essential for the property of LE cell induction (42). The antigen in question has never been well characterized, and even though 60–70% of SLE patients experiencing disease activity show a positive LE cell test, this test is not used very much anymore after the introduction of more reproducible and sensitive tests for diagnosing SLE. Anti-DNP antibodies mostly produce a homogeneous pattern in indirect immunofluorescence (Fig. 3).

*Antibodies to soluble nuclear ribonucleoprotein (nRNP) and Smith antigen (Sm)*

The two antigens nRNP ( = Mo) and Sm are easily extracted from nuclei by physiologic buffers and were therefore called extractable nuclear antigens (ENA) (74, 101), but the recognition of many nuclear antigens which are soluble in physiologic buffers (21, 158) makes the expression ENA for nRNP and Sm less suited.

Both antigens are complexes between small RNA's and polypeptides which together form RNP particles, and there is some evidence of a physical association between the two antigens (103, 125), although Sm also may exist as separate molecules (27, 63, 125). Antibodies to the antigens were originally detected in sera from SLE patients (74, 158) and the names anti-Mo and anti-Sm refer to names of patients in whom the antibodies were first described.

Anti-nRNP and anti-Sm precipitate small RNAs, the former reacting with U1 RNA and the latter with U1, U2, U4, U5 and U6 RNA which consist of about 100 to 200 bases (88). These RNAs are contained in separate RNA-protein complexes, seven 12 to 35 kD proteins being complexed to U1 RNA and five of these proteins being complexed to U2, U4, U5 and U6 RNAs of Sm (85). Anti-nRNP only react with the U1 RNP, whereas anti-Sm react with all five RNPs. A more precise name would therefore be anti-U1 RNP and anti-U1, U2, U4, U5, U6 RNP antibodies. However, if extraction of nuclear antigens is done in SDS directly, anti-nRNP sera predominantly react with a 70 kD nuclear protein and to some extent with a 31 kD and a 19 kD protein, whereas anti-Sm-containing sera strongly recognize a 25 kD doublet protein as well as a 13 kD protein (62, 63, 64). Further studies have identified the 70 kD and the 25 kD doublet proteins as nuclear matrix proteins. The U1 RNP particle exhibits two antigenic sites, one which is sensitive to RNA-ase treatment and one which is not.

Recently, the U1 RNP was shown to be complementary to the splicing junctions of heterogeneous nuclear RNA (hnRNA) and to function in the splicing of mRNA (87). In a cell-free system anti-nRNP were found to block this mRNA splicing (186). The recent suggestion by Alarcon-Segovia et al. that possibly IgG anti-nRNP might cause cellular defects if they enter living cells via Fcγ-receptors, may be mediated through blocking of mRNA formation (5, 7). The penetration of living cells by ANA, however, needs to be confirmed in studies from other scientists.

Antibodies to nRNP and Sm were initially demonstrated by precipitation techniques, but later a sensitive passive hemagglutination technique was developed (143). Both antibodies produce a speckled nucleoplasmic staining pattern by indirect immunofluorescence (Fig. 5).

Among the rheumatic diseases, anti-Sm of the IgG class are practically only found in SLE (126, 158). In a mixed population of American SLE patients anti-Sm are found in about 20–30%, but in acute SLE anti-Sm may be present in 75% of the patients (144, 158). The prevalence of anti-Sm antibodies in SLE patients in Western Europe may be considerably lower (about 15%) than in American patients (95). IgM antibodies to Sm and RNP may commonly be found in patients with parasitic infec-

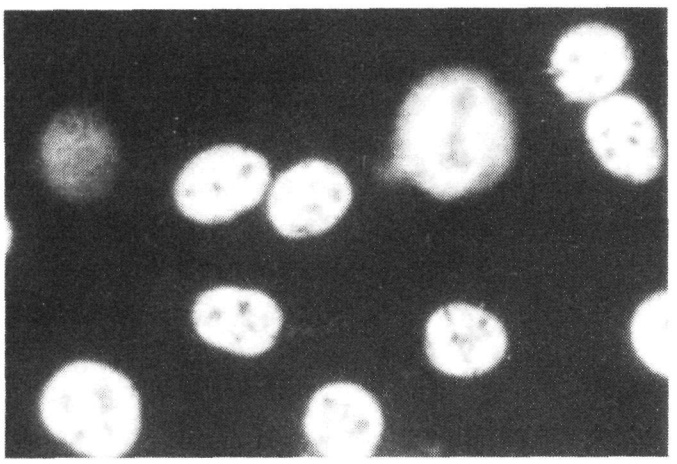


Fig. 5. Granular nucleoplasmic staining by anti-nRNP antibodies (HEp-2 cells).

tions such as schistosomiasis and malaria (188), indicating that snRNP structures are highly conserved antigens of the eukaryote nucleus. Recent studies of the malaria parasite support this assumption (52). Anti-Sm positive SLE patients may be prone to develop cutaneous and visceral vasculitides (16), but they rarely get diffuse glomerulonephritis or central nervous system involvement (120, 183). However, not all investigators have reached similar conclusions (118).

The Sm antigen binds very strongly to DNA and thus may interfere with the reactivity between anti-DNA antibodies and DNA, whereby the renal glomeruli may be protected from development of immune complex damage (115).

In contrast to anti-Sm, antibodies to nRNP may be found in many inflammatory rheumatic diseases (126, 158), but they seem to be especially characteristic for patients with overlap syndromes between SLE, polymyositis (PM), arthritis and scleroderma (171). Such overlap cases were designated "mixed connective tissue disease" (MCTD) by Sharp et al. (143). Presence of anti-nRNP was originally included in the definition of MCTD, but later on several "sero-negative" cases of MCTD were described who later developed the antibodies. In MCTD the anti-nRNP hemagglutination titers are usually extremely high and may reach $> 10^6$. When a large number of patients with connective tissue diseases are studied for anti-nRNP, positive cases have three predominant clinical features: swollen fingers, Raynaud's phenomenon, and synovitis (133). Polymyositis and fibrosing alveolitis may be other features related to high anti-nRNP titers (34, 171).

In patients with SLE, anti-nRNP antibodies are most often found in conjunction with a number of other specific ANAs, while in MCTD they are usually the only demonstrable ANA specificity, i.e. the two diseases have very different ANA profiles (95, 158). Other diseases showing presence of anti-nRNP may also have characteristic ANA profiles, suggesting that spectra of ANAs rather than single specificities should be looked for when diagnosing inflammatory rheumatic diseases (61).

It may be valuable to follow the levels of anti-nRNP in overlap syndromes, since a rise in concentration has been found to precede major flares of disease activity (63, 75).

### *Antibodies to SS-A and SS-B (Ro and La, Ha) antigens*

Antibodies to Ro were first described in 1969 in patients with SLE combined with Sjögren's syndrome (SS) (35). Such antibodies were later shown to be identical to anti-SS-A (10) which 4 years earlier had been found in a similar group of patients (13).

The SS-A (Ro) antigen is found in cells of human spleen, liver, kidney, lung and lymph nodes and is enriched in the cytoplasmic fraction upon subcellular fractionation (35). Indirect immunofluorescence studies have indicated that SS-A may be a nuclear antigen (Fig. 6), but the exact location of the antigen has not yet been settled (11, 92). It is richly represented in extracts of human B lymphoblastoid cells lines such as Wil2 cells (11), but it was also recently isolated from human spleen as a 55 kD protein that is relatively heat stable, insensitive to RNA-ase and trypsin treatment but sensitive to papain digestion (169). The SS-A (Ro) protein is associated with 75–112 base RNAs called Y1 RNA, the function of which is presently unknown (86). The SS-A/Ro particle is likely to be physically associated with the SS-B (La) antigen (169).

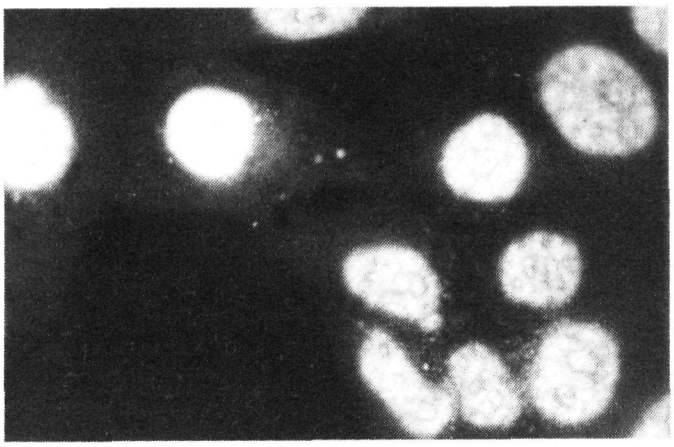


Fig. 6. Fine granular nucleoplasmic and nucleolar staining by anti-SS-B antibodies (HEp-2 cells).

Antibodies to the La antigen were originally identified in patients with SLE and SS (102). The antigen was thought to be of RNP nature because of its sensitivity to RNA-ase and trypsin digestion. Later studies, however, have shown that the antigen is insensitive to RNA-ase treatment and that solely the protein part of the complex is responsible for the reactivity with autoantibody (86). The La antigen was found to be identical to the Ha antigen (4) which had a molecular weight of 43 kD, and to the SS-B antigen (10) described 4 years earlier (13). Immunofluorescence studies indicate its presence in the nucleoplasm with a speckled distribution very similar to SS-A (86, 126, Fig. 6). It is not known why the SS-B antigen isolated biochemically by Teppo et al. seemed to exist in a higher molecular weight form of 68 kD (162). A 50 kD SS-B protein seems to be a short-lived precursor of the 43 kD protein (45).

It is particularly interesting that the protein part of SS-B, which in uninfected cells associates with several small snRNAs, strongly binds to virally encoded small RNAs such as EBER 1 and EBER 2, which are products of the EBV genome, and to VA I and VA II RNAs encoded by the Adenovirus genome. These SS-B/viral RNA complexes are strongly reactive with anti-SS-B antibodies (89). A common factor linking these viral RNAs together could be their transcription by RNA polymerase III (138). Very recent observations indicate that it is not the SS-B protein itself but a protein associating to the SS-B/snRNA complex which actually is the RNA polymerase III transcription factor (60), and Kurilla & Keene have forwarded the hypothesis, that the SS-B antigen may participate in the switch between cellular RNA production and viral replication (82).

Precipitating anti-SS-A are found in about 30 % of SLE patients, probably indicating a subpopulation with photosensitive skin disease, positive rheumatoid factor tests and symptomatic or latent Sjögren's syndrome (91, 116). Anti-SS-A may have a pathophysiologic role in the development of lupus nephritis, evidenced by the finding of enriched concentrations of anti-SS-A in glomeruli of lupus nephritis patients (94). The anti-SS-A level increases just before or in conjunction with nephritis exacerbations (141). About 53 % of SLE patients harboring anti-SS-A but not anti-SS-B have or will develop nephritis, whereas SLE patients producing both antibodies rarely (9 %) develop this manifestation (173). The difference may partly be explained by the higher frequency of anti-DNA in anti-SS-A positive SLE patients (116, 173). Anti-SS-A may contribute strongly to the hypergammaglobulinemia of SLE (93).

Neonatal lupus erythematosus, which is characterized by photosensitive dermatitis and often congenital heart block, is strongly related to anti-SS-A in the mother and her newborn child, and the dermatitis disappears concomitantly with the antibodies in the children (135, 174). Anti-SS-A transfer through the placenta is also related to congenital heart block occurring in children of asymptomatic mothers and to non-SLE connective tissue diseases (135, 142). Anti-SS-A may have a direct pathogenetic role in congenital heart block (68). Patients whose clinical symptoms are consistent with the diagnosis of SLE but lack ANA on routine testing, so-called "ANA-negative" SLE, form a small part of SLE cases (2–5 %). Two thirds of these patients produce anti-SS-A, and many of them anti-ssDNA as well (92). Photosensitive dermatitis is the main manifestation of these cases, which are often rheumatoid factor positive. The dermatitis may actually take the form of subacute cutaneous LE (SCLE), which is a non-scarring annular or papulosquamous skin erup-

tion possessing a characteristic histologic picture (149). These SCLE patients have a high prevalence of the HLA DR3 alloantigen which has been shown to be linked to the presence of anti-SS-A (17). About 75 % of patients with homozygous C2 and C4 deficiency and SLE have been found to harbor anti-SS-A (174).

The highest frequencies of anti-SS-A are found in patients with primary SS (80–100 %) where they mostly appear together with anti-SS-B (67, 174). High titers are said to be found in SS cases with pronounced extraglandular disease activity, such as neutrophilic vasculitis, purpura and leucopenia (9, 112). However, the very frequent coexistence of anti-SS-A and anti-SS-B in primary Sjögren's patients and the parallel serum level fluctuations of these antibodies in conjunction with disease exacerbations (141) makes it difficult to draw conclusions as to which antibody(ies) is most closely connected with extraglandular disease activity. Presence of anti-SS-A antibodies in RA patients may designate a subpopulation having early SS and a special propensity for developing side effects to D-penicillamine (117).

Anti-SS-B antibodies are most frequently found in patients with primary SS (70–95 %), and thus may be a diagnostic marker of this disease. However, the frequent occurrence of anti-SS-B in SLE with secondary SS found by some investigators (4, 102, 170) may decrease its diagnostic value for primary SS. Other authors, however, have found low frequencies of anti-SS-B in SLE with secondary SS (11, 116, 141, 175), so this question is still open. All authors agree that anti-SS-B antibodies are rarely found in SS secondary to RA, PSS, primary biliary cirrhosis and MCTD. It is interesting to note that the finding of anti-SS-B in patients with systemic rheumatic complaints but paucity of sicca symptoms may lead to an early diagnosis of primary SS (96, 158). Serologic abnormalities such as hypergammaglobulinemia and presence of rheumatoid factors and/or anti-SS-B in patients presenting with simple keratoconjunctivitis may also help the ophthalmologist recognize early cases of primary SS (50). In addition to the precipitation techniques and immunofluorescence technique, sensitive ELISA assays are now available for quantitation of anti-SS-A and anti-SS-B (67, 170, 185).

*Antibodies to proliferating cell nuclear antigen (PCNA) = cyclin = PL-5*

Anti-PCNA are directed to a nuclear antigen which is enriched or selectively present in proliferating cells, particularly in the late G1 and the early S-phase (111, 154). It has recently been established that PCNA is identical to antigen Ga (100) and to cyclin (97), a 36 kD protein which first accumulates in nucleoli and then migrates into the nucleoplasm during cell proliferation (154). It can be found in a few cells in bone marrow smears from patients with lymphocytic and myeloid leukemias (148). This protein may have a role in the control of cell proliferation related to DNA replication (33). Anti-PCNA are practically only found in SLE (3 %), possibly primarily in cases showing pronounced lymphoproliferation (158). Anti-PCNA are routinely screened for by indirect immunofluorescence on HEp-2 cells (Fig. 7), but recently a sensitive ELISA technique has been developed (127).

*Antibodies to scleroderma-70 (Scl-70) antigen = DNA topoisomerase I, Scl-A = RNA polymerase I, Scl-B = PM-Scl, and Scl-C antigens.*

Precipitating antibodies to Scl-70 antigen were originally described in patients with PSS (41). The antigen was isolated from rat liver nuclei by KCl extraction of chromatin, and under those conditions the isolated material appeared as a basic non-histone protein of 70 kD molecular size. The protein was sensitive to pronase and trypsin but not to DNA-ase and RNA-ase treatment. Precipitating anti-Scl-70 antibodies were found in about 20 % of PSS patients (156) being highly specific for this diagnosis (32, 158). However, if anti-Scl-70 antibodies were revealed by ELISA technique (59) one fourth of patients with SLE were found to have non-precipitating anti-Scl-70, possibly directed to epitopes which are different from those recognized by the precipitating antibodies (58).

Recent investigations have indicated that the Scl-70 antigen is a degradation product of a large

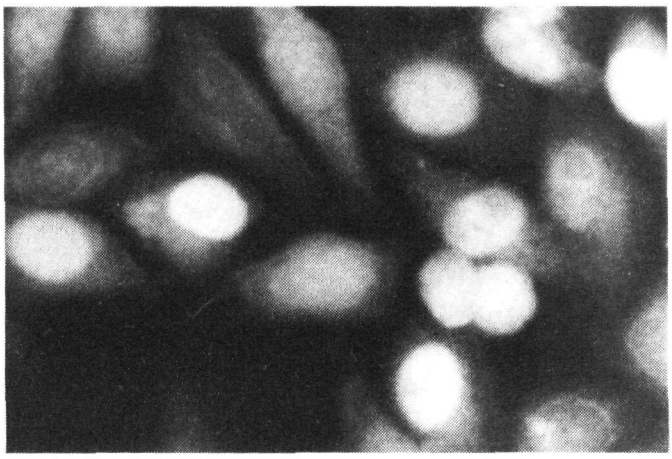


Fig. 7. Anti-PCNA (HEp-2 cells).

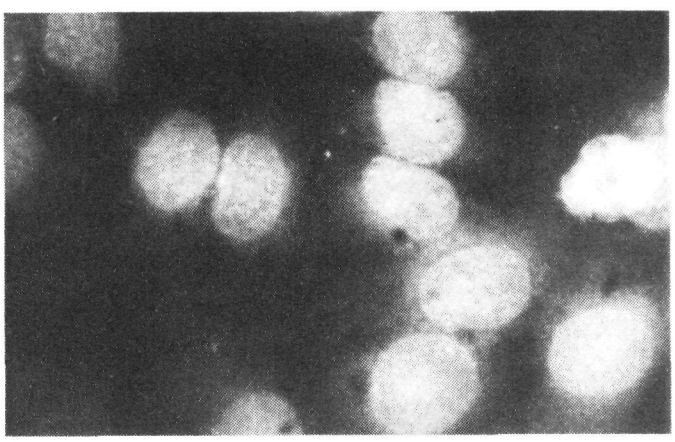


Fig. 8. Anti-Scl-70 (HEp-2 cells).

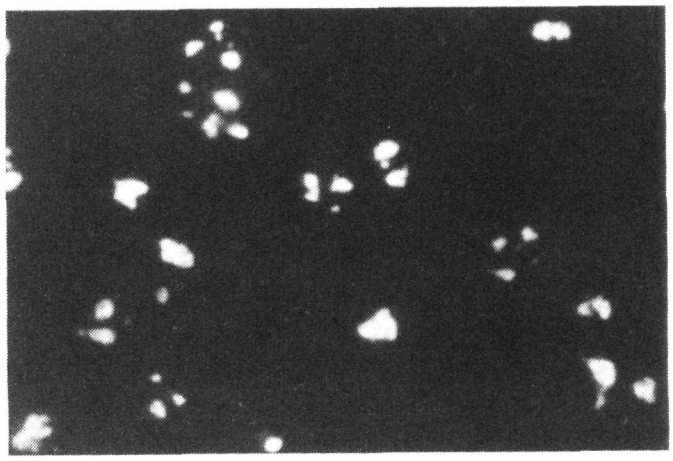


Fig. 9. Anti-nucleolar antibodies (HEp-2 cells).

water insoluble nuclear matrix protein, the extraction of which demands the use of boiling sodium dodecyl sulphate (168). The matrix protein was judged to be 86 kD in size, but later studies indicate a size of about 100 kD (8). All sera containing anti-Scl-70 antibodies reacted with the Scl-86/100 kD antigen (8, 168). Anti-Scl matrix antibodies demonstrated by immunoblotting technique were found to be a rather common marker of the diffuse form of PSS, being found in about 60 % of these cases as opposed to only about 10 % of limited scleroderma cases (168). The demonstration of anti-Scl-86 in patients with Raynaud's phenomenon may signal the ultimate development of diffuse PSS (168). Anti-Scl-70 antibodies can be visualized by immunofluorescence technique as a diffuse grainy nuclear and nucleolar staining (Fig. 8). Very recent studies have identified the antigen as being DNA topoisomerase I (145), which functions in the homeostatic control of chromosomal supercoiling.

As mentioned earlier, about 50 % of scleroderma patients harbor antibodies to nucleolar antigens. Antibodies producing a speckled nucleolar staining pattern (24, Fig. 9) (anti-Scl-A) recognize some 13 protein bands in SDS-PAGE and react with fibrillar centers of the nucleolus as shown by immunoelectron microscopy. The antibodies appear to be directed towards RNA polymerase I and designate a subgroup of scleroderma patients having diffuse systemic sclerosis (137).

Antibodies producing a homogeneous nucleolar staining pattern (anti-Scl-B) mostly recognize seven to eight proteins with a size distribution between 20 and 100 kD, identical to the PM-Scl antigen (137). Anti-PM-Scl antibodies are found in overlap syndromes between scleroderma and polymyositis (see later).

Antibodies producing a clumpy nucleolar staining pattern (anti-Scl-C) are directed to a 34 kD dimethylarginine rich protein forming particles with U3 RNA (90). The function of this RNP is as yet unknown and specific clinical correlates must await further studies. Scleroderma sera may also contain antibodies to nucleolar 7-2 RNP (134).

*Antibodies to centromere (kinetochore).*

Within the spectrum of PSS a particular constellation of clinical symptoms has been delineated because of its peculiarity and relatively benign prognosis, the CREST syndrome (Cal-

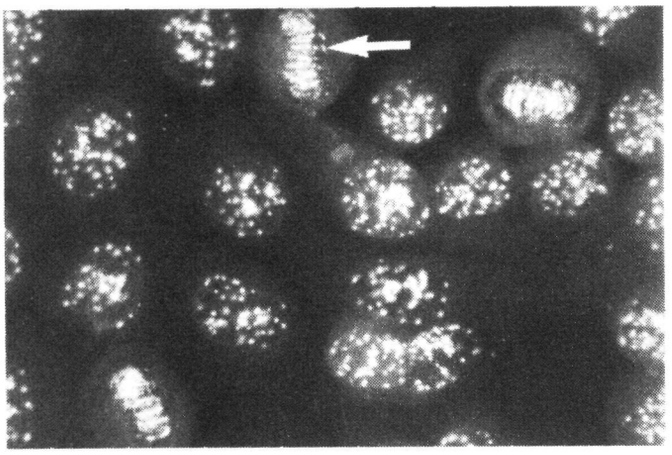


Fig. 10. Anti-centromer antibodies (HEp-2 cells). Note staining of mitotic plate region in dividing cells (arrow).

cinosis, Raynaud's phenomena, Esophageal dysmotility, Sclerodactyly and Teleangiectasia) (57, 156). The sclerodermal changes are usually limited to the hands, feet and face, and diffuse scleroderma rarely develops. Patients with this particular syndrome produce a very characteristic ANA directed towards antigens in the centromere region of the chromosome, visible by immunofluorescence on chromosomal spread preparations as two small centromere dots, corresponding to the location of the kinetochores of the centromere (113, 158). In interphase cells these antibodies produce a discrete nuclear staining pattern (Fig. 10), and in metaphase cells the speckles are lined up on the metaphase plate. The antigen is a 19 kD protein which is insoluble in physiologic buffers but soluble in sodium dodecyl sulphate (168), tightly bound to DNA of the kinetochore (113) and located on the inner and outer plates of this (26). In interphase cells the kinetochores predominantly associate with nuclear and nucleolar membranes (114). The name anti-CR-19 has recently been proposed for anti-centromere antibodies (64), however, recent studies by Earnshaw et al. have identified two additional centromere proteins of 80 and 140 kD size which are also recognized by anti-centromere antibodies (43), so this name may be inappropriate.

Anti-centromere antibodies are very characteristic for the CREST variant of PSS (24, 57, 105), being found in 60 to 90% of these patients, who all seem to have limited scleroderma that progresses slowly and never or rarely develop renal manifestations. However, the generally favorable prognosis may be disturbed, since some of the patients will develop decreased oxygen diffusing capacity and pulmonary hypertension. The finding of anti-centromere antibodies in patients with Raynaud's phenomena, but otherwise limited or absent signs of connective tissue disease, may indicate later development of the full CREST syndrome (57). As yet no studies have indicated any relation between antibody levels and clinical disease activity. It is thought-provoking that anti-centromere antibodies have been found also in patients with primary biliary cirrhosis (PBC) and incomplete CREST manifestations, since the antibodies may reflect systemic involvement at an early stage of the disease and predict which particular manifestation pattern will develop later on. Experience from our own laboratory indicates a very high diagnostic specificity of anti-centromere antibody for the CREST syndrome: among 415 patients with various connective tissue diseases 35 patients were found to harbor anti-centromere antibodies, 33 of them fulfilling at least four of the five criteria for CREST (132).

*Antibodies to the nuclear/cytoplasmic antigens: PM-1, PM-Scl = PL-6, Jo-1 = PL-1, PL-7, Pl-12, Mi-1, Mi-2 and Ku = Ki = p70/p80 in polymyositis (PM)*

Specific precipitating antibodies to soluble nuclear antigens were described in sera of myositis patients in 1977 (184), at first thought to be directed to a single nuclear antigen, PM-1, but later this was found to represent several nuclear protein antigens (158). Antibodies to the most important antigen, PM-Scl, react strongly with three 26-36 kD proteins which are present in the nucleoplasm and in the nucleolus (136).

In the original communication Wolfe et al. found anti-PM-1 in 64% of patients with PM but only in 17% of dermatomyositis (DM) patients (184). Cases having overlapping features to PSS nearly always had the antibody. Anti-PM-Scl = Scl-B = PL-6 are especially

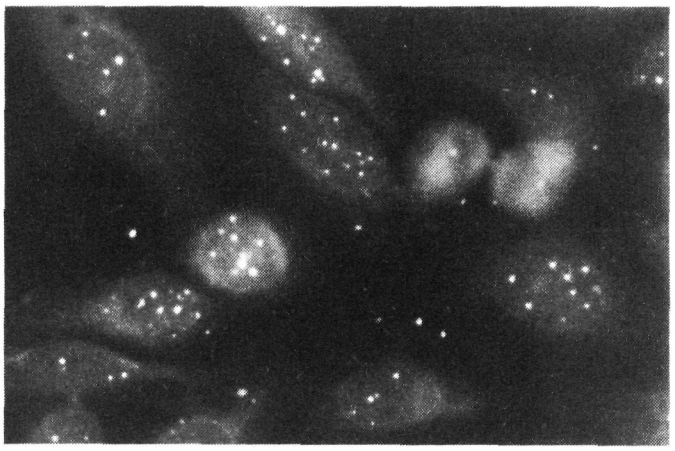


Fig. 11. Nuclear dots staining (HEp-2 cells).

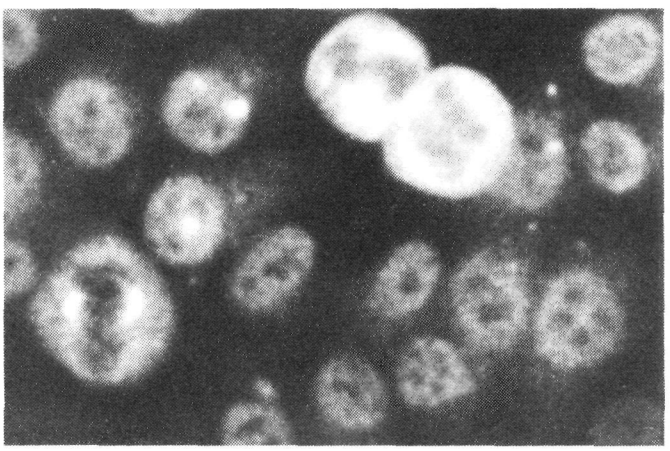


Fig. 12. Anti-mitotic spindle apparatus antibodies (HEp-2 cells).

found in PM patients with fibrosing alveolitis (108, 164). Immunofluorescence technique shows predominance of PM-Scl in nucleoli.

In 1980 Nishikai & Reichlin demonstrated a heterogeneous pattern of precipitins against calf thymus nuclear and whole cell extracts in PM and DM (123), altogether in 65 % and 59 % of these groups, respectively. Antibodies were most frequently directed to an antigen which was named Jo-1. This was present both in nuclear and whole cell extracts as a heat labile, trypsin-sensitive but DNA-ase- and RNA-ase-resistant protein estimated to be 150 kD in molecular size from gel filtration experiments. Later studies have shown that Jo-1 is a 50 kD protein of enzyme nature, histidine tRNA synthetase, the activity of which is blocked by anti-Jo-1 antibodies (99). The Jo-1 antigen may form complexes with different tRNA species, and it has been hypothesized that a particular anti-Jo-1 response is the reaction against a picorna virus RNA complexed to the synthetase (99). Immunofluorescence test with immunoaffinity concentrated anti-Jo-1 antibody suggested that Jo-1 is a nuclear antigen, but recent investigations have indicated its presence also in the cell cytoplasm (45, 64). 25–30 % of patients with pure PM have anti-Jo-1 antibodies in their serum, but PM cases showing fibrosing alveolitis, Raynaud's phenomenon, sicca syndrome and arthritis harbor it more often (about 70 %) (19). These findings have been confirmed (108, 172). The Jo-1 antigen is identical to the PL-1 antigen described by Bunn (28).

Antibodies to PL-7, a 80 kD enzyme, threonine tRNA synthetase (98) are found in 2–5 % and anti-PL-12 antibodies, directed to alanine tRNA synthetase (20) in about 1 % of PM cases.

Antibodies to the antigen Mi-1, a 150 kD basic nuclear protein, have been detected in 10 % of DM patients (124). This antigen-antibody system has received little attention diagnostically and may in fact be more characteristic for SLE than for DM (159). Antibodies to the antigen Mi-2, a nuclear protein judged to be 53–61 kD in molecular size and altogether antigenically different from Mi-1, are very characteristic of patients with DM, being found in 20 % (160). The functional roles of the Mi-1 and Mi-2 antigens are as yet unknown.

Antibodies to the Ku antigen, a 300 kD nuclear acidic protein, were originally described in 55 % of patients with PM showing overlap to PSS, in 33 % of PM and in PSS/SLE overlap but rarely in SLE or PSS without overlap manifestations (109). Later studies, however, have cast doubt about the relationship of anti-Ku antibodies to PM/PSS syndromes showing their presence in anti-Sm positive cases of SLE (53). The Ku antigen exhibits immunological identity with the Ki and the g70/g80 antigens (53, 110). The antibodies produce a diffuse speckled nuclear and nucleolar fluorescence pattern on HEp-2 cells.

*Other ANA specificities*

Antibodies to a nuclear 32 kD acidic protein antigen called Sicca-lupus antigen (SL) were

originally demonstrated in 6% of SLE and less often in RA, MCTD and PBC (70).

SLE patients experiencing particularly severe disease with nephritis may harbor antibodies to Pl-4, a 150 kD nuclear protein (21). Antibodies to the acidic nuclear antigen Ma are found in about one fifth of SLE patients, predominantly in those featuring severe disease manifestations (182). The antigen is different from the PL-4 antigen and is located in a granular fashion along the periphery of the nucleus.

Antibodies to the 96 kD phosphoprotein PL-8 have only been detected in SLE (21). Even more rare are antibodies to PL-9, a triad of nuclear proteins of 65, 67 and 86 kD (21).

Antibodies directed towards the 140 kD nuclear protein kinase N II, which functions in the activation of RNA polymerase I, may similarly be found in SLE, but also in MCTD (151).

About 4% of patients with SLE produce antibodies to the 150 kD acidic nuclear protein Su, which is located in a speckled fashion in the nucleoplasm (165).

Antibodies recognizing a 43 kD nuclear protein in proliferating cells produce a pattern of nuclear dots (Fig. 11) in immunofluorescence (24). They may be found in several autoimmune diseases (21).

Antibodies to 60–70 kD lamin proteins of the nuclear envelope may be found in patients with linear scleroderma (107). Antibodies to the mitotic spindle apparatus (Fig. 12) are very rare, and their diagnostic specificity is uncertain (69, 104).

### *Conclusion*

The collective data presented in this review appear to indicate that specific ANA determination has become an important tool in diagnosing and prognosing a number of inflammatory rheumatic diseases. ANA serology may to some extent help the clinician place his patients into clinical subgroups developing characteristic manifestation spectra within the major diagnostic entities which are usually based on certain sets of disease criteria. These subgroups may have very different therapeutic needs. Increasing levels of certain ANA's may predict major flares of disease activity and thus alert the clinician to the need for early prevention or therapy. Some ANA may represent fingerprints of viral infections, indicating that the old question about viruses as possible causative agents in initiating autoimmune diseases is still open. Except for the participation of some nuclear antigens and ANA's in immune complex-mediated inflammatory processes, little is known about the possible pathogenetic roles of ANA unless more data confirm the finding of IgG ANA penetrating into living cells to induce functional disturbances in the cells involved. Many of the nuclear antigens mentioned in this review have been or are being biochemically characterized.

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