Antinuclear antibodies: cause of disease or caused by disease?

Antinuclear antibodies (ANA) are (auto)antibodies that are reactive with antigens in the nucleoplasm. These antibodies probably occur in the circulation of all human beings, but the employed test is only considered 'positive' if they occur at titres elevated significantly above the normal serum level. ANA were first demonstrated in 1957 by Holborow et al., using indirect immunofluorescence [1]. After more than 40 yr, this method is still used as a screening technique, although the employed substrate has evolved from organ tissue to cultured cells. Since the molecular characterization of (most) antigens, other techniques, such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting, have been developed that allow the precise identification of many ANA specificities. The more precise characterization of the involved antigens has also taught us that some ANA actually react with antigens that do not predominantly occur in the nucleus, but more in the cytoplasm or on the membrane of the cell. Yet, the term 'antinuclear antibodies' continues to be used for these antibodies.

Elevated levels of ANA are found in all systemic rheumatic diseases, with sometimes high, sometimes rather loose associations between a particular ANA specificity and a particular rheumatic disease. Therefore, the detection and identification of ANA has gained increasing acceptance by clinicians who use the information to help or confirm a diagnosis and in treatment follow-up. In this Editorial, we will focus on the role of ANA in the pathogenesis of rheumatic disease, centred around the question 'Are ANA caused by the disease or the cause of the disease'. First, however, we need to have a closer look at the antigens involved.

Most ANA are directed against nucleic acids or proteins associated with nucleic acids. In systemic lupus erythematosus (SLE), the most predominant antigen is probably the nucleosome.

Nucleosomes form the basic structure of chromatin and have an important function in the compaction of DNA in the nucleus of the cell. A nucleosome consists of dimers of the four core histones, H2A, H2B, H3 and H4, which together form a histone octamer around which 146 bp of DNA are wrapped twice. Two nucleosome subunits are connected via a stretch of linker DNA to which histone H1 is bound. ANA reactive with DNA, especially if measured by a sensitive and specific radioimmunoassay (ELISA), are considered the hallmark of SLE. Antibodies to histones (and associated ubiquitons) also occur, and more recently, the existence in SLE of nucleosome-specific antibodies (i.e. recognizing a conformational epitope formed by DNA and histones) has been proven.

Another nuclear particle of importance as an antigen for ANA is the so-called snRNP (small nuclear ribonucleoprotein) particle. These particles, built from a capped small nuclear RNA molecule (U-RNA) and a number of polypeptides, have an important role in messenger RNA processing. Mammalian cells contain more than 14 different U-RNAs and more than 25 different polypeptides have been identified as constituents of the major snRNP particles. Of these proteins, the Sm proteins (BB, D1/D2/D3, E, F and G) form important antigens for ANA. Other relevant snRNP antigens are the 70 kDa, A and C proteins.

Nuclear antigens also include specificities such as RA-33, Ku, PCNA, and nuclear enzymes. For a more precise description of these, see [2]. Of the more cytoplasmic antigens, the La/SS-B protein functions as a termination factor for RNA polymerase III [3]. Transcripts of RNA polymerase III (such as Y-RNAs, 7S RNA, 5S RNA, tRNA, certain viral RNAs) to which La/SS-B is bound form the so-called small cytoplasmic RNP (scRNP) particles. Ro/SS-A also binds to Y-RNAs, so Ro/SS-A and La/SS-B are often present on the same scRNP particle. In human cells, two different Ro/SS-A molecules have been identified, designated Ro52 and Ro60. About 30% of both Ro/SS-A and La/SS-B is localized in the nucleus of the cell, which explains the nuclear immunofluorescence of anti-Ro/SS-A and anti-La/SS-B.

Other cytoplasmic antigens of relevance for ANA binding are rRNP (ribosomal RNP) and cytoplasmic enzymes such as aminoacyl tRNA synthetases (see [2]). At the time patients are diagnosed as having a rheumatic disease, ANA are generally already present. For certain ANA (e.g. anti-DNA and anti-Scl-70) it has even been shown that their presence in the circulation may precede overt clinical disease by more than 1 yr [4, 5]. So it seems a legitimate question to ask is whether such antibodies indeed mediate the disease features.

Traditionally, ANA were considered to play an active role in disease, since SLE was considered to be an immune complex disease. In this concept, ANA bind to their respective antigens, either in the circulation or in situ, and the resulting immune complexes deposit in the tissues, where subsequent complement activation leads to inflammation and disease features. Especially in the case of anti-DNA, deposition of DNA/anti-DNA complexes was supposed to be implicated in the induction of lupus nephritis. In later years, this concept was modified, based on studies that have shown anti-DNA to interact with tissue structures such as heparan sulphate, the major glycosaminoglycan side chain of the glomerular basement membrane [6, 7]. More recently, such interactions have been shown to be mediated by nucleosomal material [8].
The concept of anti-DNA playing a direct role in the pathogenesis of SLE is based on much circumstantial evidence. The various pieces of evidence pointing in the direction of an active role in pathogenesis are:

1) Anti-DNA fluctuates in time, in close association with exacerbations and remissions of the disease: especially nephritic exacerbations are heralded by an increase in the level of anti-DNA, while anti-DNA levels drop steeply during the clinical exacerbation [9]. In fact, upcoming exacerbations can be prevented by treatment of patients on the basis of increasing levels of anti-DNA [10].

2) Patients that do not have SLE at the time anti-DNA is first detected in their circulation generally develop SLE within the next 5 yr [4].

3) Antibodies to DNA can be eluted from affected kidneys [11, 12].

4) Perfusion of rat kidneys with histones, DNA and antibodies to DNA leads to the binding of anti-DNA to the glomerular basement membrane [13]. Initially thought to be based on anti-DNA cross-reactivity, we now know this binding is mediated by nucleosomes.

Taken together, these pieces of evidence indicate that anti-DNA is directly implicated in the induction and propagation of inflammatory reactions in affected tissues.

In longitudinal studies in patients with SLE or Sjögren’s syndrome, no relationship between serum levels of anti-Ro/SS-A and anti-La/SS-B antibodies and clinical symptoms and/or disease exacerbations could be demonstrated [14, 15]. In contrast, in the same patients (SLE group only) anti-DNA activity correlated very well with disease activity. These findings also implicate that anti-Ro/SS-A and anti-La/SS-B responses are regulated independently from anti-DNA. Anti-Ro/SS-A and anti-La/SS-B responses generally correlate with one another, implicating a central role for scRNP particles in eliciting and sustaining the anti-Ro/SS-A and anti-La/SS-B responses [14]. Yet, the absence of a correlation with disease activity defies a crucial role in the pathogenesis of the disease.

An exception to this may be found in the relationship between the presence of anti-Ro/SS-A (and anti-La/SS-B) and the occurrence of isolated congenital heart block (CHB) in their offspring. ANA pass the placenta and remain in the circulation of the child for several weeks after birth. The presence of ANA in the fetal circulation may give rise to neonatal lupus syndrome in the child, and, in some cases the presence of anti Ro/SS-A (and anti-La/SS-B) leads to CHB. Anti-Ro/SS-A (present in up to 98% of mothers of CHB patients) was reported to form the largest single risk factor for the development of CHB in the child [16]. Yet, the reported risk of having a child with CHB for anti-Ro/SS-A-positive mothers is only about 5%. This risk increases to about 20% in subsequent pregnancies. It is of interest that when (non-rheumatic) mothers of children with isolated CHB were studied in the setting of the cardiology department of a children’s hospital, all mothers were found to have anti-Ro/SS-A (and most of the time also anti-La/SS-B) in their circulation [17].

Regarding the mechanism of induction of CHB, it has been suggested that anti-Ro/SS-A and/or anti-La/SS-B react with their corresponding (auto)antigens expressed on the surface of cells of the fetal cardiac conduction system [18]. Indeed, anti-Ro/SS-A has been eluted from affected fetal heart tissue [19]. Other proposed mechanisms include cross-reactivity (of anti-La/SS-B) with fetal laminin, or the existence of a special fetal form of Ro/SS-A (alternative splicing).

These studies indicate that anti-Ro/SS-A and maybe anti-La/SS-B are indeed involved in the pathogenesis of CHB. Yet, in adults, the antibodies seem to be caused by ‘disease’ only: they are also found in apparently healthy women (mothers of CHB children) and in rheumatic patients do not correlate with disease features.

A correlation between SLE disease exacerbations and levels of anti-U1-RNA in the circulation has been demonstrated by Hoet et al. [20]; yet, whether this implies a direct role of this antibody specificity in the pathogenesis of SLE remains to be elucidated.

Of the other ANA specificities, some were found to correlate weakly with disease features (or severity), but no overt roles in the pathogenesis of rheumatic diseases were shown. The absence of disease correlations was recently extended to anticardiolipin (including anti-beta2-glycoprotein I and antiprothrombin) antibodies in patients with SLE or the antiphospholipid syndrome [21].

As discussed above, certain ANA specificities seem to be directly involved in the pathogenesis of disease (e.g. anti-DNA, anti-Ro/SS-A and maybe anti-U1-RNA), yet most ANA seem not. This would suggest these ANA to be caused by disease rather than to be the cause of disease. Actually, even though some ANA play a direct role in disease features, this still does not explain their existence. This brings us to the question ‘What causes ANA production’.

It is the current view that autoantigens themselves drive the autoimmune response against them. In this view, nucleosomes are probably the most relevant autoantigens for the genesis of antibodies to nucleosomes, histones and DNA. T cells with nucleosome specificity have been identified in human and murine lupus [22]; these T cells may not only be involved in the induction of antinucleosome antibodies, but also of anti-DNA antibodies. Burlingame et al. [23] have shown that antinucleosome antibodies precede anti-DNA in time in MRL/lpr mice. In SLE, increased levels of nucleosomes have been identified in the circulation [24]. Our own results (to be published) even show nucleosome levels to be inversely related to anti-DNA levels.

With regard to ANA such as anti-Sm, anti-U1-RNP/U1-RNA, anti-Ro/SS-A and anti-La/SS-B, the snRNP and scRNP particles might be the antigens involved. A possible clue to the question how nuclear antigens get exposed to the immune system comes from work by Casciola-Rosen et al. [25], who reported the
presence of nucleosomes and other nuclear antigens in surface blebs of cells dying from apoptosis. Indeed, apoptosis has in recent years been implicated in autoimmune disease. Several hypotheses regarding the relationship between apoptosis and autoimmunity have evolved from these studies:

1. A defect in apoptosis may lead to incomplete elimination of autoreactive T cells in the thymus. In MRL/lpr mice both anti-DNA production and resulting SLE-like disease are brought about by a defect in CD95 (Fas), a receptor implicated in apoptosis [26]. So far, defects in Fas or its ligand have not been found to be involved in autoimmune disease in man.

2. Prolonged increased apoptosis (due to a combination of inherited traits and environmental factors) may lead to increased levels of apoptotic cells, blebs, or autoantigens in the circulation. Indications of increased apoptosis in SLE have been found in the increased levels of soluble Fas; these levels are the highest in patients bound to develop an exacerbation [27]. Also, as mentioned above, serum nucleosome levels inversely correlate with disease exacerbations.

3. Prolonged increased levels of autoantigens in the circulation may, on the other hand, also result from improper elimination of apoptotic material; e.g. a defect in clearance mechanisms could lead to autoimmunization. Inherited deficiencies of early factors of the complement system often present with SLE. Early complement factors are important for the clearance of immune complexes, but also play a role in the clearance of apoptotic cells [28]. Indeed, mice made deficient for C1q by gene targeting develop ANA and glomerulonephritis [29].

Serum amyloid P component (SAP), a highly conserved plasma protein named for its universal presence in amyloid, binds specifically to chromatin under physiological conditions by displacement of histone H1. This leads to solubilization of chromatin, which is otherwise insoluble in plasma. Furthermore, SAP binds in vivo both to apoptotic cells, the surface blebs of which bear chromatin fragments, and to nuclear debris released by necrosis. SAP may therefore participate in the clearance of chromatin exposed by cell death. Recently, it was shown that mice with targeted deletion of the SAP gene spontaneously develop ANA and severe glomerulonephritis [30]. These findings indicate that SAP, mediating the clearance of nuclear material, prevents the formation of pathogenic autoantibodies against chromatin and DNA.

4. Autoantigens present in blebs of apoptotic cells are very sensitive to oxidative challenge or other modifications. This may lead to modifications in the autoantigens, rendering them immunogenic to the autologous immune system [31].

Due to defects in apoptosis itself or to defects in clearance mechanisms involved with the elimination of apoptotic material, nuclear antigens may become exposed to the immune system and become antigenic. These defects alone may indeed lead to autoimmune disease, such as in C1q-deficient patients with SLE, or may predispose for autoimmune disease. In the latter case, environmental factors such as (viral) infections may then be the trigger that launches the autoimmunity, and, following from that, autoimmune disease, in such persons.

Once the autoantibodies are present in a person, some play a direct role in the pathogenesis of the disease (e.g. anti-DNA in SLE and anti-Ro/SS-A in CHB), but most just seem to be the result of the disease and, as such, be considered epiphenomena of the disease.

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