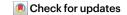
Review article



Pathogenesis of autoimmune disease

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Supplementary Box 1 | Autoantibody assays

The assay of autoantibodies confronts many technical issues that can influence the utility of serological determinations in routine care. For some autoantibodies, available assay kits can differ in the basic immunochemical principles. For example, assays for autoantibodies to proteins can use antigens that have been immunochemically purified from tissues or derived as recombinant products from in vitro expression systems. In some cases, an assay may involve a fragment of an antigen or even a peptide that has been specifically designed on the basis of increased antigenicity. A peptide called cycle citrullinated peptide (CCP) is the most common antigen used to assay for antibodies to citrullinated proteins (anti-citrullinated protein/peptide antibodies (ACPAs)) — a marker of rheumatoid arthritis. In addition to differences in antigen preparation, the format of assays can differ. Enzyme-linked immunosorbent assays (ELISA) and LINE immunoassays enable detection of specific antibodies but can differ in their ability to quantify antibody levels — an important consideration when using an autoantibody as a biomarker for disease activity. A popular format for autoantibody testing is called an ALBIA (addressable laser bead immunoassay), which allows the simultaneous detection of antibodies bound to a panel of antigens for "multiplex" determinations. It is not unusual for testing laboratories to offer different formats for detection of the same autoantibody. Understanding of techniques used in an assay will better enable a clinician in assessing the performance characteristics of an assay (for example, its specificity and sensitivity) and its capacity for quantitation. Technology in this area is rapidly evolving; the future will likely see much more extensive use of multiplex assay with an even greater number of features.

Supplementary Box 2 | Molecular features of nuclear antigens

Nuclear molecules are common targets of autoantibodies in patients with rheumatic disease. As a group, these autoantigens show the following features: they exist as complexes of proteins and nucleic acid; they are expressed in all cells; they are essential for cell function; they are positively or negatively charged; they undergo degradation or modification upon cell death; they are able to translocate into the extracellular space; they can exist in particles (extracellular vesicles); and they demonstrate immunological activity¹. The binding of autoantibodies to these molecules can be assessed in terms of models or "rules" of antigenicity derived from studies on immunization with proteins or peptides, including the results of vaccination of humans; similar studies can characterize antigenic determinants recognized by antibodies arising during infection. The application of these "rules" to autoimmunity is uncertain in view of the underlying immune disturbances of affected individuals as well as the conformation and accessibility to the immune system of these antigens as they occur in vivo. Among autoantigens, nuclear molecules display a unique set of molecular features that may lead to immunogenicity and, correspondingly, determine the autoantigenic sites recognized by antibodies.

Supplementary Box 3 | Antinuclear antibody assays

Tests for antinuclear antibodies (ANAs) are probably the most commonly ordered test to screen for the presence of an autoimmune disease and therefore have potential utility in detecting preautoimmunity. The principles of the test are well established and involve the determination of the binding of antibodies in a patient serum to a cell line called HEp-2, which is of epithelial origin. The binding of antibody is detected by use of a fluoresceinated anti-immunoglobulin agent for visual inspection; computer programs for digital imaging have been developed to reduce subjectivity. Results are reported in terms of the pattern of antibody binding (e.g., homogeneous, speckled) and titer. These patterns result from the intranuclear location of target antigens and may be important in identifying the target antigen and the disease association. Thus, a homogenous pattern may indicate the presence of antibodies to DNA and a diagnosis of SLE; similarly, antibodies producing a centromere pattern may point to limited cutaneous systemic sclerosis². An important issue concerns terminology since this test can also detect antibodies to cytoplasmic molecules. Formally, antibodies to cytoplasmic molecules are not antinuclear antibodies but they may nevertheless be clinically significant. If, despite the presence of cytoplasmic staining, a serum is called negative, the ordering clinician may not be alerted to the possibility of autoimmunity. To highlight the significance of cytoplasmic staining, "anti-cell antibodies" or "anti-HeP2 cell antibodies" have been suggested as alternative terms. Although the antinuclear antibody test is venerable and informative, its use in the clinic remains problematic because of lack of standardization or harmonization. The biggest problem with the antinuclear antibody test, however, relates to the frequency of positive results of the otherwise healthy population. This frequency can range to as high as 20%, with women having a greater frequency of positive values than men. In the face of a positive value, many laboratories will perform "reflex" testing involving other assays as a multiplex ALBIA or LINE assay for antibodies to nuclear antigens. Even if "reflex" testing is negative, the significance of the results is unclear. Given the importance of pre-clinical autoimmunity to fundamental research as well as screening for early treatment, a better understanding of the antinuclear antibody test is essential since it will likely remain among the common tests for the presence of autoimmune disease (and pre-clinical autoimmunity) for the foreseeable future³.

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- 2. Damoiseaux, J. *et al.* Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. *Ann. Rheum. Dis.* **78**, 879-889, (2019).
- 3. Pisetsky, D. S. & Lipsky, P. E. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* **16**, 565-579, doi:10.1038/s41584-020-0480-7 (2020).