

Progress report

Mitochondrial antibodies (AMA)

Ten years after recognition of the mitochondrial immunofluorescence pattern and its association with primary biliary cirrhosis¹, the value of this test has been validated and repeatedly confirmed²⁻⁷. These antibodies have been detected in 84 to 96% of patients with primary biliary cirrhosis and the minor differences between published series can be accounted for by technical details. Most workers think it preferable to test sera at 1:10 since undiluted specimens may give some non-specific fluorescence and occasionally strongly positive sera show prozone effects and appear negative until diluted. The use of monospecific anti-IgG conjugate probably increases the diagnostic accuracy but also reduces the number of positive cases of primary biliary cirrhosis.

In cryptogenic cirrhosis, the patients who have these antibodies are usually middle-aged women, often with features of cholestasis and biopsies showing certain similarities with primary biliary cirrhosis^{2,5}. Similarly, some patients with active chronic hepatitis have these clinical affinities with primary biliary cirrhosis and typical mitochondrial reactions of comparable titres^{5,8,9}. More recently another subgroup of patients with active chronic hepatitis has been recognized where immunofluorescent reactions, although resembling the mitochondrial tests, have proved to be due to antibodies directed against a membrane antigen of the rough endoplasmic reticulum which is in the microsomal cytoplasmic fraction^{10,11}. These active chronic hepatitis patients appear to be quite different from the subgroup who show mitochondrial antibodies in that the sex incidence is approximately equal, they are much younger, and cholestasis is not a feature¹². They also differ from patients with 'lupoid' hepatitis in that they show little or no antinuclear or smooth muscle reactivity.

It has also been appreciated that AMA occur in a small percentage of patients with various collagenoses and in the idiopathic autoimmune disorders of the thyroiditis/gastritis/adrenalitis group. Thus the clinical coexistence of primary biliary disease with collagen disorders, particularly rheumatoid arthritis, systemic sclerosis¹³⁻¹⁷, the CRST syndrome (calcinosis, Raynaud phenomenon, scleroderma, and telangiectasia)¹⁴ and with Sjögren's syndrome¹⁷ and thyroiditis⁹ has been well reported. However, a subclinical form of liver disease has also been found in a proportion of patients with these disorders selected on the basis of a positive AMA reaction¹⁸⁻²⁰. This form of hepatitis is characterized by lesions akin to both primary biliary disease and active chronic hepatitis. In addition, two systemic lupus erythematosus-like syndromes without liver involvement have been identified, where mitochondrial antibodies are a feature. One showed a neuropathy resembling multiple sclerosis but with SLE-like serology and a biological false-positive Wassermann reaction²¹. In these patients mitochondrial antibodies were usually of low titre and of IgM class. More recently, another curious syndrome akin to systemic lupus erythematosus associated with mitochondrial antibodies

has been delineated by German authors^{22,23}. The patients were mostly middle-aged women who presented with recurrent fever, pleural effusions, joint or muscle pains, with a high ESR. Unlike classical systemic lupus erythematosus, none of these cases had renal involvement or skin rashes, and they tended to have a leucocytosis rather than leucopenia. Serologically they showed high titres of AMA which were almost confined to the IgG class, while LE-cells and ANA were absent. Although liver function tests were mostly within normal limits, about 10% of these patients showed periportal lymphoid infiltrates on liver biopsy and one third of the cases had raised serum IgM levels, as seen in primary biliary cirrhosis.

Serological overlap between primary biliary cirrhosis and the collagenoses is also manifested by the presence of ANA in some 25% of cases of classical primary biliary cirrhosis and SMA in about 45%²⁴. . . . usually in trace amounts. However nuclear antibodies of high titre may be present in the few clinically and histologically typical primary biliary cirrhosis cases showing no mitochondrial reactivity. This could represent a counterpart to the systemic lupus-like syndrome with AMA described by the German authors.

Aside from these disorders, several large epidemiological studies have confirmed the rarity of AMA in the general population. In several countries, an incidence of 0.4 to 0.7% has now been reported²⁵. Also, in cirrhosis of types other than those already discussed, and in other forms of cholestasis, most importantly in that due to extrahepatic biliary obstruction, most authors agree that the incidence is almost equally low. It should be appreciated that there is an increased incidence of gallstones in cirrhosis^{26,27}, including primary biliary disease²⁸. One group of workers have reported persistent high titre AMA in prolonged cholestasis due to various causes^{29,30}, including main duct obstruction, in contradiction to several published series spread over a nine-year period. Others have studied certain difficult cases where there was incontrovertible evidence of extrahepatic block but where relief of the obstruction did not cause diminished production of mitochondrial antibodies, and in some cases at least there was an underlying intrahepatic inflammatory process related to primary biliary cirrhosis⁹. One explanation for the reported positive AMA in extrahepatic biliary disease may be found in the number of immunofluorescence patterns which may be produced in the tissues of different species with human sera³¹. Thus at least six discrete and unrelated reactions may resemble those produced by mitochondrial antibodies on rat kidney and stomach sections, which are frequently used as substrates for AMA tests.

In the context of sera from liver patients, the most important reaction to be differentiated is that produced by the microsomal antibodies described earlier¹⁰. These react preferentially with liver and kidney where they stain mainly the third part of the proximal tubules in the rat, while AMA produce their main reactions on distal tubules in the medulla³². For a time it was mistakenly thought that this proximal tubular pattern was attributable to a second mitochondrial antibody found in cases of active chronic hepatitis²⁴, but this pattern has now been definitely identified as the liver/kidney/microsomal antibody.

Other patterns resembling those due to AMA are unconnected with liver disease. They include a ribosomal fluorescent antibody found in less than 1% of patients with systemic lupus erythematosus³³ and which stains kidney

tubules diffusely, the so-called brush-border heterophile antibody which also reacts with rat gastric parietal cells^{34,35} and occurs in 3% of normal people³⁶. Another class of antibodies which is known to have caused confusion in inexperienced hands are the heterogeneous reticulin antibodies³⁷ and vascular endothelial antibodies³⁸. Lastly, various other patterns may be seen by immunofluorescence on rat kidney sections, due to antibodies that have not yet been properly investigated and whose clinical significance is obscure³⁹.

An antibody which can be called mitochondrial since it is directed against cardiolipin, the diphospholipid responsible for the Wassermann reaction, which is located entirely in the mitochondrial inner membrane, sometimes occurs in a fluorescent form and is found mainly in secondary syphilis⁴⁰ and in a few patients with monoclonal gammopathies and biological false positive reactions⁴¹. This antibody produces a similar fluorescence pattern to that caused by AMA but which can be easily absorbed with cardiolipin. It is unassociated with liver disease. The antigen reacting with primary biliary cirrhosis sera is also located in mitochondrial inner membranes^{42,43} but is chemically different and behaves like a lipoprotein.

Prior claims that mitochondrial antibodies are present in a high proportion of patients with halothane jaundice cannot be substantiated and the incidence does not exceed 2% in hepatic reactions due to other drugs^{2,5}.

The origin of mitochondrial antibodies is still largely unexplained. It has been convincingly shown that the HB hepatitis virus is unassociated with primary biliary cirrhosis or those conditions where AMA occurs^{44,45}. There is, however, evidence of a familial predisposition to primary biliary cirrhosis and related disorders^{46,47}, while the symptomless relatives of subjects with primary biliary cirrhosis have an incidence of AMA of 7 to 8%^{48,49} which is about ten times that found in the general population. Despite the negative correlations with HBAg, however, it is still quite possible that some other virus or related particle (? icon) plays a causal role in autoimmune disorders⁵⁰. It is even conceivable that in primary biliary disease some phage-like organism invades the mitochondria in bile duct cells, since in a different context it has been suggested that mitochondria can become infected with virus-derived nucleic acids⁵¹.

It has been shown that the lymphocytes of patients with primary biliary cirrhosis produce migration inhibition factor when cultured in contact with mitochondrial antigen⁵² as with a biliary protein⁵³. This could be considered as the cellular limb of the immune reaction against mitochondria which is such a prominent feature in these patients, in the same way as thyroiditis patients show a positive leucocyte migration inhibition test with thyroid microsomes⁵⁴ to which they have antibodies, and pernicious anaemia patients react with intrinsic factor⁵². However the situation with regard to the mitochondrial leucocyte migration inhibition test is far more complex. It appears that patients without any evidence of serum AMA or of liver disease, but who suffer from idiopathic autoimmune disorders such as thyroiditis⁵⁵, gastritis, adrenalitis, or even a disease where autoimmunity is inconspicuous, namely, insulin-sensitive diabetes⁵⁶, also give positive migration inhibition when their leucocytes are cultured in the presence of liver mitochondrial inner membranes. Normal people do not show this phenomenon and outer mitochondrial membranes do not produce this effect⁵⁷. It thus appears as if autoimmune diseases in general might be associated with a cellular immunity to a mitochondrial component, possibly through cross reaction between

infecting organisms to which they may be atavistically related⁵⁸.

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