Section of Clinical Immunology & Allergy

President E J Holborow MD

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President's Address

Smooth-muscle Autoantibodies, Viral Infections and Malignant Disease

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What significance smooth-muscle antibody has in disease is a problem that illustrates only too well the often-encountered difficulty of attaching plain clinical meanings to the presence of autoantibodies in patients' sera. Our contributions towards a solution have depended more on the kindness of several different collaborators in making their clinical material available, than on the pursuit of clues clearly recognized from the start. Nevertheless, the sequence of facts we have uncovered so far, which began with a chance finding of no more than moderate relevance to the diagnosis of liver disease, is now perhaps extensive enough to indicate that in smooth-muscle antibody we are studying an autoimmune response of general biological as well as of clinical interest.

The initial chance observation (Johnson *et al.* 1965) was that on cryostat sections of rat stomach, used as substrate to test patients' sera for antibody to gastric parietal cells by indirect immunofluorescence, occasional sera produced bright well-defined staining of fibres running linearly between the gastric glands, which we identified as smooth muscle fibres.

Sera giving this staining also stained the smooth muscle fibres of the muscularis mucosæ, of the muscle coats of the stomach, and of the smooth muscle coat of arteries, and we established that the staining was due to an antibody, predominantly IgG, that reacted specifically with smooth muscle fibres and not with skeletal or with cardiac muscle fibres. Sera with enough of this smooth-muscle antibody (SMA) to give bright unmistakable staining at titres up to 1/100 or more proved usually to have come from patients with chronic active or aggressive hepatitis, whose liver biopsies showed the characteristic infiltration of the portal tracts with lymphocytes and plasma cells, with destruction of adjacent parenchymal cells and accompanying fibroblastic response. The association of SMA with chronic active hepatitis was confirmed by Doniach *et al.* (1966). At about the same time Walker *et al.* (1965) discovered another autoantibody, mitochondrial antibody, in the sera of patients with primary biliary cirrhosis. Largely as a result of their subsequent work, it is now generally held that mitochondrial antibody, SMA and the antinuclear antibodies, which caused a group of these patients to be labelled as cases of 'lupoid' hepatitis (MacKay *et al.* 1956), are all serological markers of a larger group of chronic liver diseases which includes chronic active hepatitis, primary biliary cirrhosis and some cases of cryptogenic cirrhosis, which together fall under the same heading of autoimmune hepatitis (Doniach & Walker 1969).

Apart from the question of what role, if any, these or other autoimmune responses play in producing the liver lesions, one of the major puzzles was why smooth-muscle antibody should occur so regularly, when smooth muscle fibres, unlike nuclei and mitochondria, are conspicuous by their virtual absence in the liver tissues directly involved. What is the antigenic stimulus that leads to the production of SMA? This question gained further force when we began to run across examples of SMA in sera from cases outside this particular group of hepatic diseases; this was in marked contrast to mitochondrial antibody of the M type, which is almost confined

to the three chronic liver syndromes I have mentioned (Doniach *et al.* 1966).

The first extensive study that we conducted outside chronic autoimmune liver disease was in acute infective hepatitis (Farrow *et al.* 1970). Dr Farrow had observed a group of patients from soon after the onset of acute infective hepatitis, and had collected specimens of their sera week by week during the course of the illness. On clinical and epidemiological grounds he assigned the patients to three categories, according to whether the probable mode of infection was oral, parenteral or unknown. On testing these sera for a range of autoantibodies, it soon emerged that SMA was present in a large majority – 80% – giving a staining pattern indistinguishable from that already known in chronic active hepatitis. SMA was present as often in the 'orally infected' group, where Australia antigen was not usually found, as in the 'parenterally infected' where Australia antigen was usually detected in the serum; SMA was most often present early in the disease, declining in frequency over a period of weeks. The SMA of acute viral hepatitis is predominantly IgM in class, and the titres seldom exceed 1/80. From this study we concluded that SMA in viral hepatitis reflects the presence of parenchymal liver cell damage rather than the presence of Australia antigen in the serum. Moreover, in chronic active hepatitis Wright (1970) found a virtually mutually exclusive relationship between Australia antigen and SMA. But the puzzle remained – why SMA?

We have been aware for some time that sera which give a pattern of smooth-muscle staining on rat stomach sections often also stain two other sites in rat tissue sections, the renal glomeruli and the liver cells themselves, outlining the latter in a polygonal pattern which we at first attributed to staining of bile canaliculi, but which we came to interpret differently as a result of observations in a different quarter, as follows.

Jones *et al.* (1970) found that when trypsin-dispersed embryo chick liver cells were exposed to rabbit antiserum raised against human smooth-muscle actomyosin extracted from parturient myometrium, the spontaneous reaggregation of these cells was prevented in specific fashion. This finding supported the proposal that cell adhesion involves the participation of actomyosin-like protein located at the cell surface. More particularly to us, however, it suggested that the polygonal pattern of staining of rat liver by SMA-positive sera might be accounted for by the presence of an actomyosin-like protein in the liver cell membrane.

We prepared monolayers of chick embryo cells by culturing trypsin-dissociated liver tissue for 2–3 days, and tested sera containing SMA for their ability to stain these cells by indirect immunofluorescence (Farrow *et al.* 1971). We found that, provided the monolayers had been pre-treated with cold iso-pentane at the temperature of liquid nitrogen, a characteristic pattern of cell staining was obtained with SMA-positive sera, which appeared to involve a microfilamentous network particularly well seen in cells that were well spread on the glass. Enhanced staining at the cell margin suggested that the network was near the cell membrane, but the fact that without iso-pentane treatment no staining was seen indicates that the antigen involved is not directly accessible at the surface of normal tissue culture cells. This microfilamentous network is not exclusively found in cultures derived from liver; a network was also stained in iso-pentane-treated cultures of human fœtal lung, for example. Furthermore, absorption of SMA-positive sera with suspensions of isopentane-treated human fœtal lung cells removed their ability both to stain rat smooth muscle and to give the polygonal pattern on rat liver; and we have since found that when human sera with SMA antibody are absorbed with extracts containing human smooth-muscle actomyosin, all three patterns of staining – smooth muscle, glomerular and polygonal – are abolished, while other autoantibodies such as antinuclear antibodies or gastric parietal cell antibodies are unaffected.

These experiments, then, provide some information towards answering the question why sera from hepatitis cases are reactive with smooth muscle. They indicate that liver cells, as well as other cells, contain a component resembling smooth-muscle actomyosin, and that this component is a constituent of a microfilamentous network probably associated with the cell membrane, but not directly accessible at the cell surface. How this component is rendered autoantigenic is still a matter for conjecture, but one speculates that viral infection of liver cells may reveal or alter components immediately beneath the cell surface so that they become immunogenic.

We had noted, meanwhile, that we were getting some positive tests for smooth-muscle antibody in patients with another viral disease, infectious mononucleosis. With the cooperation of Dr H Hempsted of the Royal Berkshire Hospital, Reading, we have recently obtained and examined sera from 82 patients clinically presenting with glandular fever. The sera we tested were mostly taken within one week of the onset of symptoms, the longest interval from onset being one month. Of sera positive in the Paul-Bunnell (PB) test for heterophile antibody, 80% were positive for SMA at titres from 1/20 to 1/320, often for both IgG and IgM, unlike the heterophile antibody

itself, which is exclusively IgM. A further 9% of the sera were positive for SMA, but only at 1/10 dilution. Ten of the sera gave only weakly positive PB tests, and of these 8 were positive or weakly positive for SMA. It was also noted that of 26 patients with an illness like glandular fever but with negative PB tests, 6 (23%) had SMA titres above 1/10 and 11 (42%) were SMA-positive at 1/10 only. I will consider this PB-negative group again later.

Hepatocellular damage occurs frequently in infectious mononucleosis, and up to 90% of cases may show abnormal levels of serum enzymes. The SMA findings suggest again that virus infections that produce liver cell damage may alter the hepatocyte membrane sufficiently to render at least one of its components immunogenic, and thus stimulate the production of an autoantibody which we recognize in immunofluorescent tests chiefly through its reactivity with smooth muscle. A viral etiology for chronic autoimmune hepatitis is only moderately supported by the available evidence, but the discovery of Australia antigen (or hepatitis-associated antigen) in serum hepatitis and of Epstein-Barr virus and its antibody in infectious mononucleosis has strongly supported the viral causation of these two diseases. It may also be mentioned here that in a recent small outbreak in Nigeria of yellow fever, a hepatitis of undoubtedly viral origin, Dr J A Smith and Dr T I Francis (1971, personal communication) found SMA at titres greater than 1/8 in 13 out of 13 patients' sera tested. However, before we can implicate hepatotropic viruses as specific agents in the production of SMA, we shall have to examine a much wider range of different viral infection, and also a range of non-viral liver diseases. In a small group of 16 rubella cases from whom we obtained both acute and convalescent sera we found only 2 with weak SMA.

The PB-negative group of patients clinically suspected as having glandular fever are of interest because such cases presumably contribute to the background level of incidence of SMA that we have encountered in various different samples of the population as a whole. In a small survey of sera from healthy blood donors, for example, we found an incidence of SMA at titres greater than 1/10 in 27 out of 114, or 23%. These sera were kindly made available to us by Dr Arie Zuckerman and Dr Pat Taylor, who had tested them for Australia antigen. We found that, among those who had proved to be silent carriers of Australia antigen (56 of the 114), both the incidence (38%) and the titres of SMA were significantly higher than in those (58 of the 114) negative for Australia antigen (SMA-positive, 10%). If silent carriage of Australia antigen can significantly increase the incidence of SMA, it is not unreasonable to suspect that carriage of, as opposed to frank infection with, some other viruses might also lead to a detectable degree of formation of SMA.

Lastly, I turn to investigations we have very recently carried out in another field in which antibodies directed at cell membrane antigens are also increasingly being reported, that of malignant disease. Dr Michael Whitehouse, of the Chester Beatty Institute, and I have examined sera taken from patients with certain forms of malignant disease before treatment, not only for antibodies reacting with tumour antigens, but also for those reacting with normal tissue components – that is, for autoantibodies. A preliminary survey (Whitehouse & Holborow 1971) has dealt with 80 patients with histologically confirmed malignant tumours, partially selected to include reasonable numbers of patients with malignant melanoma and with neuroblastoma, in both of which immune responses to tumour-specific antigens have been reported. Sera from 46 normal subjects (age range 17–79 years) were also examined. SMA at titres of 1/10 or more was found in 54 (67%) of the 80 patients, and in 9 (20%) of the 46 controls. The incidence ranged from 60% in malignant melanoma to 83% in carcinoma of the ovary. All the controls positive for SMA were under the age of 31, although half this group were over this age. In the cancer group antibody was predominantly IgG, although IgM antibody was often also present.

The antibody in the sera of patients with malignant disease appears identical with that occurring in the sera of patients with chronic active or acute infective hepatitis, or with glandular fever, and gives identical patterns of staining on rat stomach, kidney and liver, which are completely removed by prior absorption with an extract of human myometrium. Apart from SMA the only other autoantibody encountered with significant frequency in this survey of malignant disease was antinuclear antibody, which was detected in 24% of the patients, but in none of the controls.

Thus smooth-muscle antibody appears to be present in a majority of patients with malignant disease examined before treatment. Since SMA reacts with a normal constituent of the cell membrane of some cell types, the interaction of this antibody with tumour cell membranes must obviously be excluded before any antibodies reacting with tumour-specific antigens in the tumour cell membrane can be identified with certainty as such.

How might the production of SMA be related to the presence of a tumour? As we have seen, SMA has hitherto been reported chiefly in

hepatic disease, or at least in the presence of hepatocellular damage, and this might suggest that its demonstration in cancer patients is merely a manifestation of metastatic involvement of the liver. The clinical data, so far as they go, do not support this explanation, since SMA was found in 6 out of 9 patients with malignant melanomas considered to be still in the primary stage. This might also argue against the idea that mere cell necrosis is a significant factor in its production. Another possibility not yet investigated is that a change in the membrane of the malignant cell results in the exposure of an actomyosin-like antigen and leads to its production in a more immunogenic form, perhaps in a manner analogous to what I have already suggested as a possibility in certain viral infections of cells.

So far, then, we have provisionally identified the antigen against which SMA is directed as smooth-muscle actomyosin, since this antibody reacts with a component of the hepatocyte cell membrane and with some other cell membranes, and an actomyosin-like contractile protein is already known to be present at or near the cell surface. On the facts presented, it seems reasonable to postulate that autoantibody directed at smooth muscle arises in acute infective hepatitis, and in glandular fever as a result of virally-induced hepatocellular changes leading to autoimmunogenicity of this actomyosin-like membrane component. In addition, certain viral infections of other cell types may perhaps lead to production of smooth-muscle antibody, while other viral infections do not. Furthermore, the finding of SMA in such a high proportion of patients with malignant disease suggests that this same actomyosin-like component may likewise become autoantigenic as a result of membrane changes accompanying the malignant transformation of cells of many different types.

As to the biological and pathological effects on the cells concerned of this immune response to a normal membrane antigen, we have no information as yet. We have still to investigate whether cell-mediated immunity to smooth-muscle actomyosin can be demonstrated in patients with the corresponding antibody and, if so, what its effects may be. Such investigations would be especially relevant to the problem of the pathogenesis of the cell destruction seen in chronic active hepatitis; and they might also throw light on the biological role of this same immune response in malignant disease.

I began by saying that it is not always easy to attach plain clinical meanings to autoimmune phenomena; but I believe we are in a better position to attack each problem in autoimmunity when we have identified the biochemical nature and subcellular location of the antigens concerned. When that location proves to be the cell membrane, as in the case of the antigen with which smooth-muscle antibody reacts, it is an encouragement to continue exploration of the implications for immunopathology.

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Walker J G, Doniach D, Roitt I M & Sherlock S (1965) *Lancet* i, 827
Whitehouse J M & Holborow E J (1971) *British Medical Journal* iv, 511
Wright R (1970) In: Immunology of the Liver. Ed. M G M Smith & R S Williams. London; p 33

Meeting November 26 1971

The following papers were read:

Immunology of Intrauterine Infection
Dr A M Silverstein
(*Wilmer Ophthalmological Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA*)

Origin and Development of Lymphocyte Populations
Dr J J T Owen
(*Department of Human Anatomy, South Parks Road, Oxford*)

The Role of Immunological Enhancement in Tumour Growth, in the Fœtal Maternal Relationship, and as One Component Helping in Maintaining 'Allograft Tolerance'
Dr K E Hellström
(*Department of Pathology, University of Washington, Seattle, Washington 98195, USA*)

Meeting March 13 1972

A laboratory meeting was held at the Clinical Research Centre, Northwick Park, Watford Road, Harrow, Middlesex. Demonstrations were given.