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# SMOOTH MUSCLE ANTIBODY IN PATIENTS WITH WARTS

S. A. MCMILLAN AND MARGARET HAIRE

*The Laboratories, Belfast City Hospital, and Department of Microbiology and Immunobiology, The Queen's University of Belfast, Belfast*

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SUMMARY

Sera from fifty-four patients with warts were tested for the presence of commonly found autoantibodies. Smooth muscle antibody (SMA) was detected at a significantly higher level than in a control group of healthy blood donors matched for age and sex, and was in the IgM class. The SMA-positive sera gave a microfilamentous staining pattern also in the IgM class, on fixed $HEp_2$ tissue culture cells. Absorption of positive sera with smooth muscle removed both SMA staining and the anticellular staining.

## INTRODUCTION

Since smooth muscle antibody (SMA), detected by indirect immunofluorescence, was first described by Johnson, Holborow & Glynn (1965) in the sera of patients with active chronic hepatitis, this autoantibody has been found in sera of patients in the acute phase of viral illnesses (Farrow *et al.*, 1970; Holborow, Hemsted & Mead, 1973). Many cultured cells, growing so that single cells are spread out before fixation, give a filamentous intracellular network pattern of staining when treated with SMA-positive sera and fluorescein-labelled anti-human IgM conjugate (Farrow, Holborow & Brighton, 1971). This pattern may be similar to the IgM autoantibody to microtubules of cells, described by Whitehouse, Ferguson and Currie (1974), in sera of patients with infectious mononucleosis or to the IgM fibrillar anti-cellular staining pattern described by Haire (1972) in the majority of sera of children with acute measles and acute mumps infection, and in twenty-seven out of forty-three patients with multiple sclerosis (Millar *et al.*, 1971).

From experimental work it would appear that SMA represents a variable range of antibodies against different intracellular contractile proteins (Trenchiev, Sneyd & Holborow, 1974; Gabbiani *et al.*, 1973). A disturbance of cells in the body during the course of a viral infection may liberate an antigen which cross-reacts with smooth muscle, so that the induced antibody also cross-reacts with SMA antibody.

Correspondence: Mr S. A. McMillan, The Laboratories, Belfast City Hospital, Belfast BT9 7AD, Northern Ireland.

Matthews & Shirodaria (1973) found IgM antibody to the non-infected cells when sera from warts patients were tested on sections of warts, and Shirodaria observed an IgM fibrillar staining pattern when the sera were tested on cultured human embryo lung cells (Shirodaria, personal communication). Therefore we thought it worthwhile to study the common autoantibodies, including SMA, and antibodies to cultured $HEp_2$ cells in warts patients to look for further evidence for the association of SMA with viral infection.

## MATERIALS AND METHODS

### *Patients*

Sera were obtained from fifty-four patients with active common and plantar warts attending dermatological out-patients departments. The patients consisted of twenty-seven males and twenty-seven females, the majority of whom were under 30 years. The control group consisted of an equal number of age- and sex-matched healthy blood donors. Sera were stored at $-20°C$ until tested.

### *Detection of conventional autoantibodies*

The indirect immunofluorescent technique was used to detect antibodies to smooth muscle, nuclear, gastric parietal cell, reticulin and mitochondrial antigens. Initial serum dilutions at 1:10 were made in 0·01 M phosphate-buffered saline at pH 7·2 (PBS), and applied to unfixed 5 $\mu$m cryostat sections from a composite block of snap-frozen rat liver, kidney and stomach, for 20 min at room temperature. They were washed for 15 min in PBS and then treated for a further 30 min with sheep anti-human globulin conjugated with fluorescein isothiocyanate (FITC) (Wellcome Reagents, Ltd), previously absorbed with rat liver powder. The optimum staining titre of the conjugate was 1:120. After washing for 1 hr the sections were mounted in glycerol–saline and examined by transmitted light on a Leitz dialux microscope fitted with an iodine quartz lamp, a Balzer FITC-3 interference filter and a K 530 secondary filter.

Staining was scored on a + + + system: + = poor staining; + + = definite staining; + + + = strong staining. SMA can be recognized by staining of the smooth muscle fibres between gastric glands, the muscularis mucosae and the walls of renal arterioles, each of these features being scored as: 0 = no staining; 1 = doubtful staining; 2 = definite staining; 3 = brilliant staining, giving a possible total score for each serum of 9; 0–4 was regarded as negative; 5 and 6 as +; 7 as + +; 8 and 9 as + + + (Holborow *et al.*, 1973). Sera giving strong positive staining reactions were titrated, and in all tests the end-point was taken as the highest serum dilution which gave + + staining. Some positive sera also gave outline staining of liver cells ('polygonal' pattern) and/or staining of renal glomeruli.

All sera were tested under code and the immunofluorescence readings were made by two independent observers.

### *Further tests on SMA-positive sera*

Sera found to contain SMA were further examined on the same antigen using anti-human IgG or IgM antibodies conjugated with FITC (Wellcome Reagents, Ltd), at their optimum staining dilutions, 1:100 and 1:30 respectively. These sera were also tested on fixed coverslip preparations of $HEp_2$ tissue culture cells. The cells were seeded thinly so that single cells were well spread out before fixation in fresh acetone for 10 min at room temperature (Haire,

1972) or by dipping in isopentane cooled in liquid nitrogen (Farrow, Holborow & Brighton, 1971). The coverslips were air-dried and stored at −20°C in the presence of silica gel before using.

*Serum absorption*

The SMA-positive sera, diluted 1:5, were absorbed for 2 days at +4°C with a saline homogenate of a surgical specimen of a human uterus. After centrifugation at 22,400 *g* the serum was passed through a millipore filter of pore size 0·45 μm. The absorbed sera were tested in parallel with aliquots of the same specimen unabsorbed, on both composite blocks of rat tissue and on the fixed cultured $HEp_2$ cells. All three conjugates were used in these tests.

## RESULTS

The prevalence of autoantibodies in the sera of patients with warts and in the control subjects is shown in Table 1, and the individual titres of SMA are shown in Fig. 1. There

TABLE 1. The incidence of autoantibodies in the sera of patients with warts and in the sera of normal healthy blood donors*

| Type of autoantibody | Study group | |
|---|---|---|
| | Number of patients with warts (%) | Number of blood donors (%) |
| Smooth muscle | 14 (25·9) | 4 (7·4) |
| Antinuclear | 2 (3·7) | 4 (7·4) |
| Anti-reticulin | 3 (5·5) | 4 (7·4) |
| Gastric parietal cell | 1 (1·8) | 1 (1·8) |
| Number in group | 54 | 54 |

* Sera were tested at 1:10 dilution.

was a significantly higher prevalence of SMA in the sera of the wart group compared with the control group ($\chi^2 = 5{\cdot}0625$; d.f. = 1; $0{\cdot}05 > P > 0{\cdot}01$, a correction for small numbers was made), and these were equally distributed between the sexes. There was no significant difference in the prevalence of antinuclear, gastric parietal cell and anti-reticulin antibodies between the same groups. The prevalence of these latter three autoantibodies was in the same order as that found in a survey of autoantibodies detected in the sera of four hundred healthy blood donors (McMillan and Haire, unpublished data). When the SMA-positive sera were further tested using FITC-conjugated anti-human IgG and IgM conjugated, the antibody was found to be of IgM class. On analysis of clinical notes of the patients there was no relationship between the presence or absence of SMA and the site or number of the warts.

When our SMA-positive sera, from both patient and control groups, and several of the SMA-negative sera from both groups were tested on the cultured $HEp_2$ cell antigen, all of

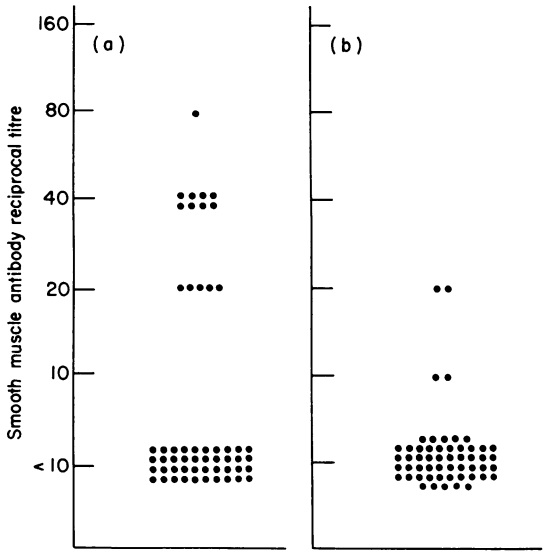


FIG. 1. The titres of smooth muscle antibody in the sera of (a) fifty-four patients with warts and in the sera of (b) fifty-four normal healthy blood donors.

the positive sera gave a strong 'fibrillar' or microfilamentous network type of staining similar to that which we have previously observed (Haire, 1972) (Fig. 2). The SMA-negative sera gave little or none of this pattern of staining. As with the SMA, this antibody was of the IgM class, and while the staining by SMA antibody and the 'fibrillar' anticellular staining were detectable with anti-human globulin conjugate, the anti-human IgM conjugate gave much brighter and more distinctive staining.

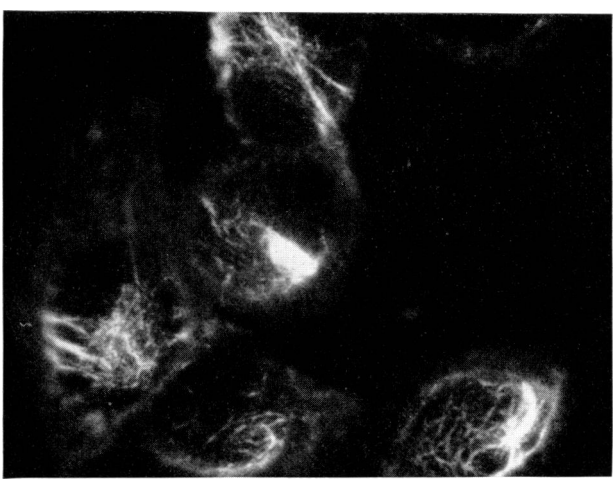

FIG. 2. Cultured $HEp_2$ cell stained with SMA-positive serum from a patient with warts and with anti-human IgM conjugated with FITC. Fibrillar pattern of staining is seen.

Absorption of sera by the preparation of smooth muscle made from human myometrium caused almost complete disappearance of both the SMA and the fibrillar staining, revealed by the two conjugates, but it did not affect antinuclear staining also present in some of the tests.

## DISCUSSION

We have shown an increased prevalence of SMA in sera of patients with warts compared with the control subjects. While this antibody is relatively common in healthy people, there appears to be no sex difference (Hooper *et al.*, 1972; McMillan & Haire, unpublished data), such as that observed with other autoantibodies, such as antinuclear, gastric parietal and anti-thyroid by Hooper *et al.* (1972) and in our own unpublished studies of antinuclear and anti-reticulin antibodies in 154 males and 154 females, matched for age. Hooper suggests that extrinsic agents, such as infection, which could affect males and females to a similar degree might in some way be responsible for this antibody, rather than an intrinsic (genetic) factor.

Fagraeus, Lidman & Biberfeld (1974) have demonstrated that SM antigen in peripheral lymphocytes increased considerably when the cells were stimulated with phytohaemagglutinin, which has probably a similar effect as a viral infection *in vivo* in releasing host cell antigens. These are then capable of stimulating an antibody which reacts with SM or cytoplasmic contractile proteins of cultured cells.

In their study of warts virus-specific immunoglobulins in patients with warts, Matthews & Shirodaria (1973) showed a prolongation of the IgM response, implying that the viral antigenic stimulus was persisting. Our finding of SMA and anticellular antibodies of IgM class implies that they are a response to a similar persistent stimulus. It would be important to make a further study to correlate the presence of anti-viral, SMA and anti-cellular antibodies in individual patients with warts.

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