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Mechanisms of smooth muscle antibody production

A clinical study in children with infections, haemolytic syndromes, and idiopathic thrombocytopenic purpura

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SUMMARY Sera from 530 children suffering from various diseases and from 64 controls were tested for smooth muscle autoantibodies (SMA) by indirect immunofluorescence. A high incidence of SMA (51-86%) was found in patients with viral and bacterial infections (viral hepatitis, infectious mononucleosis, measles, mumps, chickenpox, typhoid fever, and brucellosis), independently of liver involvement, and in patients with acute haemolytic anaemia due to G-6-PD deficiency (48%). By contrast, the incidence of SMA from patients with β-thalassaemia major and idiopathic thrombocytopenic purpura was no higher than in the controls. The discrepancy in incidence in haemolytic anaemias due to different causes may reflect the effect of endogenous and extrinsic agents. In the viral infections, SMA were mainly of the IgM class and gave an 'SMA-V' staining pattern. In bacterial infections (typhoid fever and brucellosis), SMA were either IgG only or IgM and IgG, and the staining pattern was also mainly 'SMA-V'. In infections which affect or may affect the liver (viral hepatitis, infectious mononucleosis, typhoid fever, and brucellosis), SMA was present at high titres (1:80-1:320), whereas in infections not affecting the liver (measles, mumps, and chickenpox) the titres were lower ($\leqslant$1:80). In most patients SMA occurred transiently and without apparent pathogenetic significance. The antigen against which infection-induced SMA is directed is not actin; its nature has yet to be identified.

Smooth muscle autoantibodies (SMA) are present in the sera of some patients with chronic active hepatitis (CAH) (Doniach *et al.*, 1966; Johnson *et al.*, 1965), acute viral hepatitis (Farrow *et al.*, 1970; Ajdukiewicz *et al.*, 1972; Vittal *et al.*, 1974; Kanakoudi *et al.*, 1975), infectious mononucleosis (Holborow *et al.*, 1973; Sutton *et al.*, 1974a), cytomegalovirus (Andersen and Andersen, 1975), *Mycoplasma pneumoniae* infections (Biberfeld and Sterner, 1976), and malignant disease (Whitehouse and Holborow, 1971). It has been suggested that high SMA titres (> 1/100) of the IgG class are of value in the diagnosis of CAH (Lidman *et al.*, 1976).

The nature of the antigen was first suspected by Farrow *et al.* (1971), who showed that SMA reacted with actin-like microfilaments in cultured liver cells. They suggested that viral infection of liver cells alters the submembranous microfilaments so that they become immunogenic. The antiactin specificity of SMA in CAH was subsequently confirmed by studies showing neutralisation of all antibody activity by absorption with actin from platelets or from skeletal muscle (Gabbiani *et al.*, 1973; Andersen *et al.*, 1976; Lidman *et al.*, 1976; Kurki *et al.*, 1978; Toh *et al.*, 1978). However, in diseases not involving the liver, the specificity of SMA has not been identified. Furthermore, other aspects of SMA require elucidation. First, does SMA occur in viral infections without liver involvement, or in bacterial infections which may affect the liver? Also, since some SMA are directed against actin, are these autoantibodies produced when actin is released from platelets or red cells, as in idiopathic thrombocytopenic purpura (ITP) and haemolytic anaemias? If so, are they autoimmune markers of non-immunological disease or are they related to the

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Table 1 *Clinical diagnosis and age and sex distribution of children*

Clinical diagnosis	*No. of patients*	*Sex*		*Age (years)*	
		F	*M*	*Range*	*Mean*
Viral hepatitis					
HB_sAg negative	116	40	76	2-14	7·8
HB_sAg positive	33	8	25	2-14	8·5
Infectious mononucleosis	37	15	22	1 1/2-14	6·3
Measles	49	27	22	1-12	5·5
Mumps	52	17	35	2 1/2-14	7·8
Chickenpox	19	7	12	1-14	6·5
Typhoid fever	58	29	29	2 1/2-14	9·5
Brucellosis	30	12	18	2-15	9·6
Idiopathic thrombocytopenic purpura	25	10	15	7d-15	5·5
Acute haemolysis due to G-6-PD deficiency	27	5	22	2m-10	4·0
β-thalassaemia major	84	32	52	2 1/2-16	13·4
Total	530	202	328		

immunopathology, clinical course, or prognosis of the disease?

The aim of this study was to answer some of these questions by looking for SMA in sera of children with acute viral infections, bacterial infections (brucellosis, typhoid fever), ITP, and acute or chronic haemolytic anaemia. Serum samples were obtained during the active stage of disease and one to two months after recovery. The patients positive for SMA at the second serum examination were followed up for six months to two years.

Material and methods

CHILDREN STUDIED

During a period of four years (1975-78) sera from 530 children suffering from various diseases and from 64 healthy children of similar age range and sex distribution were tested for SMA by immunofluorescence. The patients had viral and bacterial infections, ITP, and acute or chronic haemolytic anaemia. They fell into 11 diagnostic groups, and their age and sex distribution are listed in Table 1. Diagnoses were based on clinical, laboratory, and epidemiological data. Controls were from nursery and grammar school or relatives of patients hospitalised for respiratory infections in the Department of Paediatrics, University of Thessaloniki. The control children were selected on the basis of absence of overt illness for one month before blood sampling.

COLLECTION OF BLOOD SAMPLES

In patients with viral hepatitis, infectious mononucleosis, measles, mumps, chickenpox, and typhoid fever, the first serum sample was taken between the seventh and 10th day of disease, and in brucellosis patients between the 10th and 15th day. A second sample was obtained seven to 10 days after the first. When the second sample was SMA positive, a third was obtained one to two months later. In the ITP group the first serum sample was obtained on the seventh to 30th day after the appearance of a haemorrhagic skin rash and before treatment. The second sample was taken seven to 10 days later and the third two to 24 months later. In the acute haemolytic anaemia group the first serum sample was taken on the second to sixth day after the onset of clinical symptoms and before blood transfusion. The second sample was taken five to seven days after blood transfusion and the third one to two months later. In the β-thalassaemia group serum samples were obtained 25 to 30 days after the last blood transfusion. Sera were coded and stored at −20°C.

DETECTION OF SMOOTH MUSCLE AUTOANTIBODIES

Autoantibodies were detected by immunofluorescence tests on sections (4μ) cut on a cryostat at −20°C, from unfixed composite tissue blocks of snap-frozen rat stomach, liver, and kidney. Fluorescein isothiocyanate labelled sheep anti-human immunoglobulin, IgM, and IgG (Wellcome Reagents) were used at suitable dilutions. Sera were heated at 56°C for 30 minutes and tested at a 1/10 dilution. Positive sera were titrated in doubling dilutions from 1/20 to 1/320 with all three conjugates. Samples positive at dilutions < 1:40 were recorded as doubtful (Fig. 1). All patients whose second or third serum sample was positive were recalled for examination six to 24 months after recovery. Re-examination included a detailed history, clinical examination, and detection and titration of serum SMA.

IMMUNOABSORPTION STUDIES

Fifty-six serum samples were absorbed with skeletal muscle actin or myosin by the method described

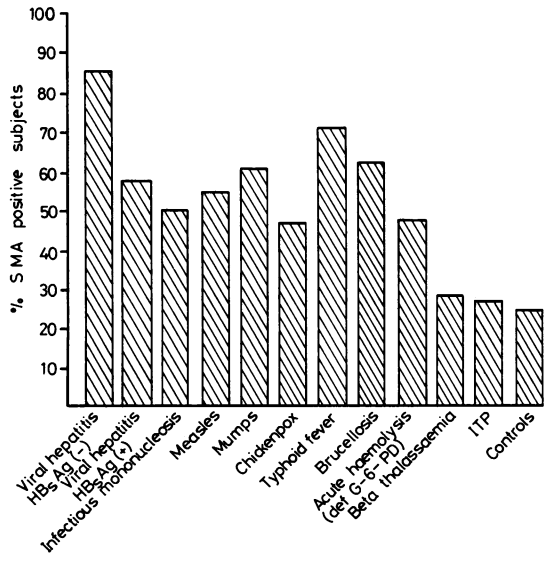

Fig. 1 *Incidence of SMA in children with various diseases and in controls.*

previously by Toh *et al.* (1978). Sera tested after absorption were from the following conditions: brucellosis (3), typhoid (3), 'G-6-PD deficiency-haemolysis' (3), infectious mononucleosis (5), viral hepatitis (16), chickenpox (8), measles (8), and mumps (8). The muscle proteins were a gift from Dr M. Owen, National Institute of Medical Research, Mill Hill, London.

Results

INCIDENCE OF SMA

Smooth muscle autoantibodies were found mainly during the acute stage of viral or bacterial infections (Table 2, Fig. 1). Compared with controls, the incidence was significantly higher in patients with viral hepatitis, infectious mononucleosis, measles, mumps, typhoid fever, and brucellosis ($P < 0.01$ or < 0.001) but not in chickenpox, ITP, and β-thalassaemia major ($P > 0.05$). The incidence of SMA was also raised in children with acute haemolysis ($0.01 < P < 0.05$).

TITRE AND IMMUNOGLOBULIN CLASS OF SMA

In most patients with viral hepatitis, infectious mononucleosis, typhoid fever, and brucellosis, SMA titres were $>1:80$, while in patients with measles, mumps, chickenpox, ITP, and β-thalassaemia major and in controls, the titres were $\leq 1:80$. In patients with 'G-6-PD deficiency-acute haemolysis' the titres were 1:80-1:160 (Fig. 2). In patients with viral hepatitis, infectious mononucleosis, measles, mumps, and chickenpox, SMA was mainly of the IgM class, but in typhoid fever and brucellosis it was IgG. In the 'G-6-PD deficiency-acute haemolysis' group, SMA was either IgG, IgM, or both. In ITP, β-thalassaemia major, and controls, SMA was mainly IgG (Table 3).

STAINING PATTERN

Most SMA positive sera stained only smooth muscle, although some also stained glomeruli (mainly sera

Table 2 *Incidence of SMA in disease and control groups*

Disease group	*SMA positive patients*			
	Positive/Total	%	χ^{2*}	P
Viral hepatitis				
HB_sAg negative	100/116	86	67·41	<0·001
HB_sAg positive	19/33	57·5	10·00	0·001<P<0·01
Infectious mononucleosis	19/37	51	7·15	0·001<P<0·01
Measles	27/49	55	10·67	<0·001
Mumps	32/52	61	15·79	<0·001
Chickenpox	9/19	47	3·53	>0·05
Typhoid fever	42/58	72	27·31	<0·001
Brucellosis	19/30	63	12·81	<0·001
Idiopathic thrombocytopenic purpura	7/25	28	0·08	>0·05
Acute haemolytic anaemia due to G-6-PD deficiency	13/27	48	4·70	0·01<P<0·05
β-thalassaemia major	24/84†	28·5	0·23	>0·05
Controls	16/64‡	25		

*χ^2 refers to comparison of various disease groups versus child controls.
†5/24 were found doubtful at 1/40 dilutions.
‡3/16 were found doubtful at 1/40 dilutions.

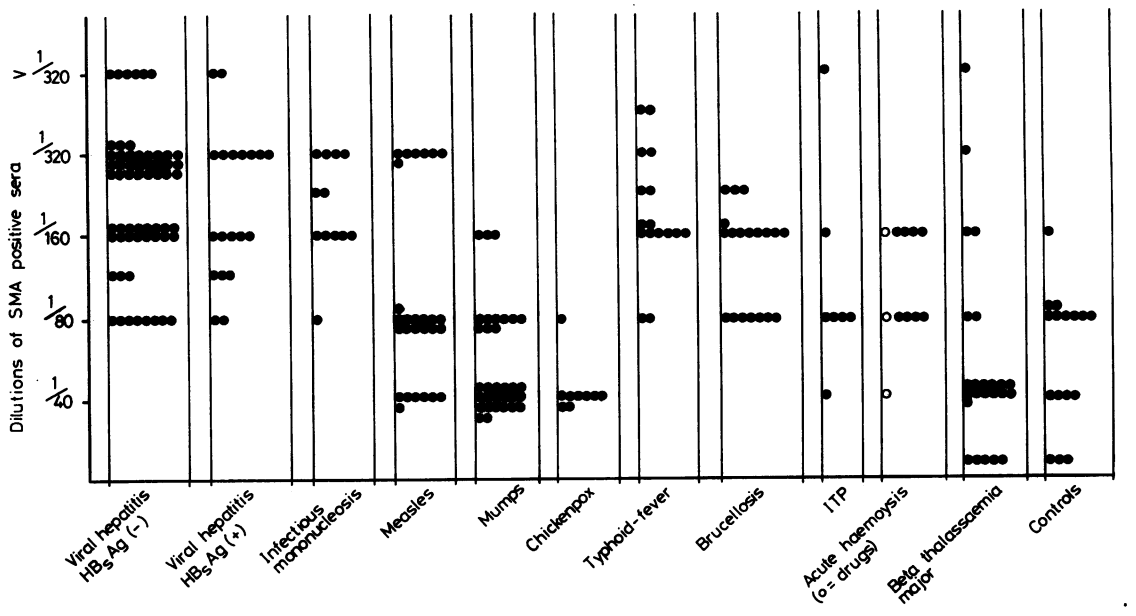

Fig. 2 *Smooth muscle antibody titre in patients and controls.*

Table 3 *Smooth muscle autoantibodies in children with various diseases: immunoglobulin class and presence in sequential serum samples*

Diagnosis	*Sequential serum samples*				*Immunoglobulin class*			
	No. tested	*1st*	*2nd*	*3rd**	*No. tested*	*IgM*	*IgM+IgG*	*IgG*
Viral hepatitis								
HB_sAg negative	116	100	65	5/40	60	45	15	0
HB_sAg positive	33	19	15	2/13	19	15	4	0
Infectious mononucleosis	37	19	10	1/9	12	3	8	1
Measles	49	27	20	1/15	15	13	2	0
Mumps	52	32	22	0/14	15	12	3	0
Chickenpox	19	9	5	0/4	9	7	2	0
Typhoid fever	58	42	23	1/13	16	0	6	10
Brucellosis	30	19	9	0/9	19	1	7	11
G-6-PD deficiency†	27	13	4	0/4	11	2	3	6
β-thalassaemia major	84	24	...	...	24	2	3	19
Idiopathic thrombocytopenic purpura	25	7	5	0/3	7	0	2	5

*Third samples from some patients whose sera were positive in the 2nd sample were also tested. The results are shown as the number positive over the number tested.
†Haemolysis due to fava beans (24) and to sulphonamides or naphthalene (3).
... Not tested.

from viral hepatitis, measles, and typhoid), and a few gave apical staining of renal tubules. These three patterns conformed with the SMA-V, SMA-G, and SMA-T of Bottazzo *et al.* (1976).

IMMUNOABSORPTION STUDIES

The SMA-V activity in 56 serum samples was not abolished by absorption with skeletal muscle actin or myosin.

LACK OF CORRELATION BETWEEN PRESENCE OF SMA AND CLINICAL AND LABORATORY FINDINGS

The results in relation to the main clinical and laboratory findings in patients during the acute stage of disease, and six months to two years later, are summarised as follows. In none of the diseases was the presence of SMA correlated with age or sex distribution of the patients. In addi-

Table 4 *Clinical and laboratory findings of patients positive for SMA 2-18 months after recovery*

Diagnosis	*Sex*	*Age (yr)*	*History*	*Transaminases*		*SMA*		*Time of re-examination (mth)*
				SGOT	*SGPT*	*Titre*	*Ig class*	
Viral hepatitis								
HB_sAg negative	F	6	Symptoms of URTI (1 wk*)	34	24	1:80	IgM + IgG	10
	M	5	,, ,, ,, (2 wk)	48	12	1:80	IgM + IgG	16
	F	11	Free of recent infection	34	6	1:80	IgG	9
	M	9	,, ,, ,, ,,	40	30	1:40	IgG	18
	M	10	,, ,, ,, ,,	48	2	1:40	IgG	6
HB_sAg positive	M	7	Free of recent infection	40	4	1:80	IgG	12
	M	8	,, ,, ,, ,,	20	4	1:40	IgG	11
Infectious mononucleosis	M	2	Symptoms of URTI (2 wk)	20	2	1:80	IgM + IgG	8
Typhoid fever	F	13	Symptoms of URTI (2 wk)	24	4	1:80	IgM + IgG	12

URTI = upper respiratory tract infection.
*weeks before coming for re-examination.

tion, in infectious mononucleosis, typhoid fever, and brucellosis, no correlation was found between the presence of SMA and liver involvement (liver enlargement, jaundice, and raised bilirubin and transaminase levels). SMA was not correlated with heterophile antibodies in infectious mononucleosis nor with severity of haemolysis in acute haemolytic anaemia. In the β-thalassaemia major group, three out of four children with SMA titres of >1:80 had had splenectomies or had a past history of viral hepatitis, but the presence of a lower SMA titre did not correlate with either of these features.

TIME OF SMA APPEARANCE AND PERSISTENCE

In most patients with infections SMA appeared during the first week and usually disappeared after six to eight weeks (Table 3). However, SMA persisted for longer than six months in five patients with HbsAg negative viral hepatitis, in two with HbsAg positive viral hepatitis, in one with infectious mononucleosis, and in one with typhoid fever (Table 4). Further investigation of these patients (detailed history, clinical examination, transaminase measurement, and SMA titration) showed that persistence of SMA was not related to the outcome of the disease or to transaminase levels, and physical examination revealed no abnormal findings. Four out of these nine SMA positive patients (2 with viral hepatitis, 1 with infectious mononucleosis, and 1 with typhoid fever) had had a history of pyrexia and upper respiratory tract symptoms in the previous one to two weeks. Their SMA classes and titres are shown in Table 4.

Discussion

A high incidence of SMA was found in children with viral and bacterial infections independently of liver involvement (51-86%) and in those with acute haemolytic anaemia due to G-6-PD deficiency (48%). By contrast, in β-thalassaemia major and ITP, the incidence of SMA was not different from that in the controls (Table 2; Fig. 1). In chickenpox, although SMA was found in about half of the patients (9/19), the incidence was not significantly raised, but this may be due to the smaller number examined. These prevalence findings for SMA in viral hepatitis and infectious mononucleosis in children are similar to those reported for adults, viz, up to 81% in viral hepatitis (Farrow *et al.*, 1970; Ajdukiewicz *et al.*, 1972; Vittal *et al.*, 1974) and up to 75% in infectious mononucleosis (Holborow *et al.*, 1973; Sutton *et al.*, 1974b).

The incidence of SMA in healthy adults, ranging from 12% to 20% (Whitehouse and Holborow, 1971; Holborow *et al.*, 1973; Sutton *et al.*, 1974a; Shu *et al.*, 1975), is similar to that in a random population sample in Greece (19·4%). A higher incidence was observed in infancy and childhood (Papastavrou *et al.*, 1979, unpublished data). In the present study SMA was present in 20% of control children at titres of >1:40 and in 25% at lower titres (<1/20). Holborow *et al.* (1973), however, found SMA in 10% of healthy children aged 10 years and under. The relatively higher incidence in the present study may be due to the wider age spectrum (infancy, pre-school age, school age, and puberty) and to the susceptibility of young infants to asymptomatic viral infections such as CMV and EBV. Another possible cause of the increased incidence of SMA in infancy may be vaccinations with live attenuated viruses which are usually performed at this early age.

The importance of extrinsic agents such as microorganisms rather than genetic factors in determining the presence and lack of sex selectivity of SMA was pointed out by Hooper *et al.* (1972), who compared the incidence of SMA with that of other autoantibodies in an Australian population.

This concept is consistent with reports of a high incidence of SMA in patients with EBV, CMV, *Mycoplasma pneumoniae* infections, and warts (Sutton *et al.*, 1974a; Andersen and Andersen, 1975; McMillan and Haire, 1975; Biberfeld and Sterner, 1976), and the present findings accord with these. Particularly interesting is the contrast between acute haemolytic anaemia due to G-6-PD deficiency and β-thalassaemia major. In both conditions, destruction of cells rich in contractile proteins occurs, but an increased incidence of SMA was demonstrated only in patients with acute haemolytic anaemia. The rate of antigen release and its amount in the circulation may be important factors for the production of SMA, but involvement of exogenous agents (ie, drugs) in the haemolysis due to G-6-PD deficiency may as well account for this difference in the findings between the two haemolytic states. SMA production under these conditions may be an example of bypass of self-tolerance by extrinsic factors, due to either cross-antigenicity with self components (Wang *et al.*, 1975) or to a 'hapten-carrier' mechanism (Allison *et al.*, 1971). The findings favour the concept that cell breakdown with release of antigens is alone insufficient to account for SMA production (Fairfax and Gröschel-Stewart, 1977).

A low prevalence of SMA was also demonstrated in ITP. The pathogenesis of this disease is not yet completely known. Classic concepts on the subject postulate that platelets react with an autoantibody and are eventually destroyed by macrophages, predominantly in the spleen (Rosse, 1978). It is not known whether the platelets are modified so that contractile protein (thrombosthenin) becomes both accessible and immunogenic (as probably happens when hepatocytes are infected by virus) nor whether antigenic material is released after destruction of platelets by macrophages so that SMA production is triggered. So far, a role for an exogenous agent which might enhance SMA production in ITP has not been established. Until these questions are settled, the low incidence of SMA in ITP cannot be adequately accounted for. However, it is worth pointing out that only acute cases responsive to corticosteroids were included in this study. It might be interesting to study chronic, cortico-resistant cases of ITP undergoing splenectomy or immunosuppressive therapy.

Antiactin specificity of SMA has been demonstrated only in CAH (Gabbiani *et al.*, 1973; Andersen *et al.*, 1976; Lidman *et al.*, 1976; Kurki *et al.*, 1978; Toh *et al.*, 1978). The present study confirms previous reports that SMA from patients with non-liver diseases does not react with actin or myosin (Andersen *et al.*, 1976; Bottazzo *et al.*, 1976; Fairfax and Gröschel-Stewart, 1977; Kurki *et al.*, 1978). Antiactin SMA in CAH is of the IgG class, persists in high titre (> 1/100), and gives a mainly 'SMA-T' staining pattern (Bottazzo *et al.*, 1976). By contrast, SMA in viral infections is mainly IgM (Andersen and Andersen, 1975), occurs transiently during the acute stage of disease, is present in low titres (< 1:80), and gives a mainly 'SMA-V' staining pattern (Bottazzo *et al.*, 1976).

Titres in viral hepatitis, infectious mononucleosis, typhoid fever, and brucellosis were high (1:80-1:320) in this study but lower in measles, mumps, and chickenpox (1:40-1:80) (Fig. 2). In viral infections SMA was mainly IgM, in bacterial infections either IgG or IgM and IgG, and in all infections was mainly of the 'SMA-V' staining pattern. The presence of IgG SMA in patients with bacterial infections may be due to serum samples being obtained later in the disease than in the viral infections (Table 3). These findings suggest that SMA are produced in high titres mainly in infections which affect or may affect the liver (hepatitis, infectious mononucleosis, typhoid fever, and brucellosis). No relationship with other clinical or laboratory findings was observed.

The early appearance and short duration of smooth muscle autoantibodies in our patients' sera emphasises that they are transient and in this respect unlike other autoantibodies such as antinuclear antibody which are often persistent markers of autoimmune disease. The lack of correlation between the presence of SMA and the clinical and laboratory findings, either during the course of the disease or after recovery, suggests that SMA do not participate in immunopathogenic processes. In the small proportion of patients in whom SMA persisted for up to 1½ years there was also no association with the course of the disease, in agreement with previous observations in viral hepatitis (Ajdukiewicz *et al.*, 1972) and infectious mononucleosis (Holborow *et al.*, 1973) patients.

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