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Abnormal humoral immune response to *Staphylococcus aureus* in patients with *Staphylococcus aureus* hyper IgE syndrome

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SUMMARY

Patients with the *S. aureus* hyper IgE syndrome (SAHIGES) have an abnormal IgE response to cell wall and surface antigens of *S. aureus*. In this paper we describe the detection of IgE antibodies to soluble antigens of staphylococci (*S. aureus* and *S. epidermidis*) and qualitative abnormalities of the IgG response to soluble *S. aureus* antigens in patients with SAHIGES. These findings may be of pathogenetic importance and help to delineate SAHIGES from other diseases.

Keywords IgG antibodies Western blot *S. epidermidis*

INTRODUCTION

The *Staphylococcus aureus* hyper IgE syndrome (SAHIGES) is characterized clinically by severe, recurrent or chronic pulmonary and cutaneous infections mainly with *S. aureus*, eczematoid dermatitis and coarse facies (Schopfer, 1984). The disease usually begins in infancy. The salient immunological features are hyperimmunoglobulinaemia E, IgE against *S. aureus* and *Candida albicans* whole cells (Berger *et al.*, 1980; Schopfer *et al.*, 1979) as well as purified cell walls (PCW) (Schopfer, Douglas & Wilkinson, 1980), abnormal regulation of IgE production by peripheral blood mononuclear cells (PBMC) *in vitro* (Geha *et al.*, 1981), decreased cell mediated immunity *in vivo*, decreased response of PBMC to mitogenic stimulation, and inconsistently impaired chemotaxis of granulocytes *in vitro* (Mawhinney *et al.*, 1980; Schopfer, 1984). The cause of immune deficiency especially to *S. aureus* is still unknown; some observations suggest a defect of cell mediated immunity (Geha *et al.*, 1981), and antistaphylococcal IgE may be of pathogenic importance. However, studies on the humoral immune response to *S. aureus* considering antigens other than surface antigens or PCW and immunoglobulin classes other than IgE have been incomplete (Dreskin, Goldsmith & Gallin, 1985). We have recently analysed IgG, IgA and IgM antibody responses to purified cell walls (Wilhelm, Matter & Schopfer, 1982). In this paper we present studies on the antibody response of SAHIGES patients using soluble antigens of *S. aureus*, *S. epidermidis* and other bacteria. We found IgE antibodies to several *S. aureus* antigens and qualitative abnormalities of the IgG antibody response in the sera of SAHIGES patients. These results raise questions on the immunopathogenic potential of this antibody response to a very common pathogen.

MATERIALS AND METHODS

Patients. Seven patients with definite SAHIGES have been described elsewhere (Schopfer,

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Table 1. Characteristics of patients

Patient	Age (years)	Sex	Diagnosis	Total serum IgE (M/ml)
1	10	M	SAHIGES	1200
2	6	M	SAHIGES	960
3	21	F	SAHIGES	15000
4	8	M	SAHIGES	24500
5	10	M	SAHIGES	6800
6	19	F	SAHIGES	1000
7	22	M	SAHIGES	8000
8	20	M	Chronic osteomyelitis	880
9	19	M	Chronic osteomyelitis	155
10	61	M	Sepsis	760
11	12	M	Chronic osteomyelitis	88
12	8	M	Subfascial abscess, furunculosis	ND

ND, not done.

1984). Five patients with severe staphylococcal infections constitute an infected control group. Some characteristics of the patients are shown in Table 1. In addition, 16 patients with asthma, rhinitis or eczema and serum IgE concentrations above 900 IE/ml (age 1 to 35 years) and four healthy male adults (age 39 to 41 years) served as controls.

Bacterial antigens. Staphylococcus aureus H, *S. epidermidis* and *Escherichia coli* were grown at 37°C for 2 to 3 days in tryptone-yeast extract with glycine and glucose. *Haemophilus influenzae* b was grown on chocolate agar for 3 days at 37°C. The suspensions were harvested at an A_{578nm} of 0·9–1·5 (dilution 1:10) and washed in phosphate buffered saline (PBS) with 5 mM iodoacetic acid, 0·5 mM phenylmethanesulphonylfluoride (PMSF) and 0·02% NaN_3 (washing buffer) at 4°C. Bacterial cells were broken in a Vibrogen cell mill (E. Bühler, Tübingen, Germany) using glass beads of 0·17–0·18 mm diameter (B. Braun, Melsungen, Germany), washed through a glass filter (Schott No. 1, Mainz, Germany) in 50 ml cold washing buffer, heated for 10 min in boiling water, cooled again on ice and centrifuged at 12,000 ***g*** for 10 min. The clear yellow supernatant was removed and frozen immediately for lyophilization. After dialysis against washing buffer, solutions were made with an A_{280nm} of 0·5.

Solid phase radioimmunoassay. Nitrocellulose sheet (Millipore) was cut into strips and 0·5 μl drops of antigen solution were spotted at even distances. After drying overnight at room temperature the strips were washed in Tris buffered saline (TBS: 0·05 M Tris, 0·15 M NaCl, pH 7·4) for 5 min; in 3 M urea with 0·1 M $MgCl_2$ for 10 min; and in incubation buffer (IB: 10 mM Tris-HCl, 0·1 M $MgCl_2$, 0·5% Tween 80, 1% bovine serum albumin, 5% fetal bovine serum) for 15 min on a rocking platform. Sera were diluted 1:2 in IB and incubated for 2 h. After two 10 min washes in IB, rabbit anti human IgE ^{125}I with a specific activity of 5 μCi/0·4 μg (RAST-reagent from Pharmacia, Zürich, Switzerland) was used for a 2 h incubation. After two 10 min washes in IB and four 5 min washes in TBS, individual dots were cut and counted in a MR 1032 Gamma counter (Kontron AG, Basel, Switzerland).

Polyacrylamide gel electrophoresis and Western blots. These were performed as described (Towbin, Staehelin & Gordon, 1979). Briefly, 1 mm thick 17·5% vertical gels were cast in a 130 × 150 mm mould. On one side a 2 cm wide slot was used for molecular weight standards (Bio Rad) and the remaining width of the gel for the *S. aureus* H soluble antigen (200 μl of a 4 mg/ml solution) in sample buffer (65 mM Tris-HCl pH 6·8, 5% mercaptoethanol, 10% glycerol, 2·3% SDS, 0·002% bromophenolblue). Electrophoresis was carried out at 20–30 mAmp for 12–15 h. The gels were then transferred into nitrocellulose sheets (Schleicher und Schüll) and blotted at 48 volts (max. 1·6 amp) for 2 h in transfer buffer (25 mM Tris, 192 mM glycine, pH 8·3, 20% methanol, 0·00004% SDS) in a

destaining bath (Zabona, Basel, Switzerland). Molecular weight standards were measured after staining with 0·1% amido black. The rest of the nitrocellulose sheet was cut in strips and kept in TBS at 4°C until used. The completeness of the transfer was checked by staining the polycrylamide gel with Coomassie brilliant blue.

The strips with the blotted antigens were reacted with sera diluted 1:2 in IB for IgE and 1:100 for IgG according to the protocol used for the solid phase RIA. For the detection of IgG, we used rabbit antihuman IgG-Peroxidase (Dako) 1:500 in IB and, after the washes, 4-chloro-1-naphthol (Merck 11 952), 3 mg/ml methanol, diluted 1:5 in TBS with 2 μl 30% H_2O_2 per 10 ml (freshly prepared) was added. The reaction was stopped after 45 min incubation at room temperature by washing three times with water.

RESULTS

Sera from all of seven SAHIGES-patients contained IgE which binds to soluble *S. aureus* antigens with molecular weights from about 200 kD to below 14 kD (Fig. 1). The pattern and the amount of IgE binding vary considerably from patient to patient. There seems to be but one common antigen for all sera, with an apparent molecular weight of about 15 kD. Only one of five sera from control patients with active *S. aureus* infection but without SAHIGES (8 in Fig. 1) has IgE binding to one single antigen of about 29 kD. All other controls including four normal uninfected persons do not make detectable IgE antibodies to these antigens.

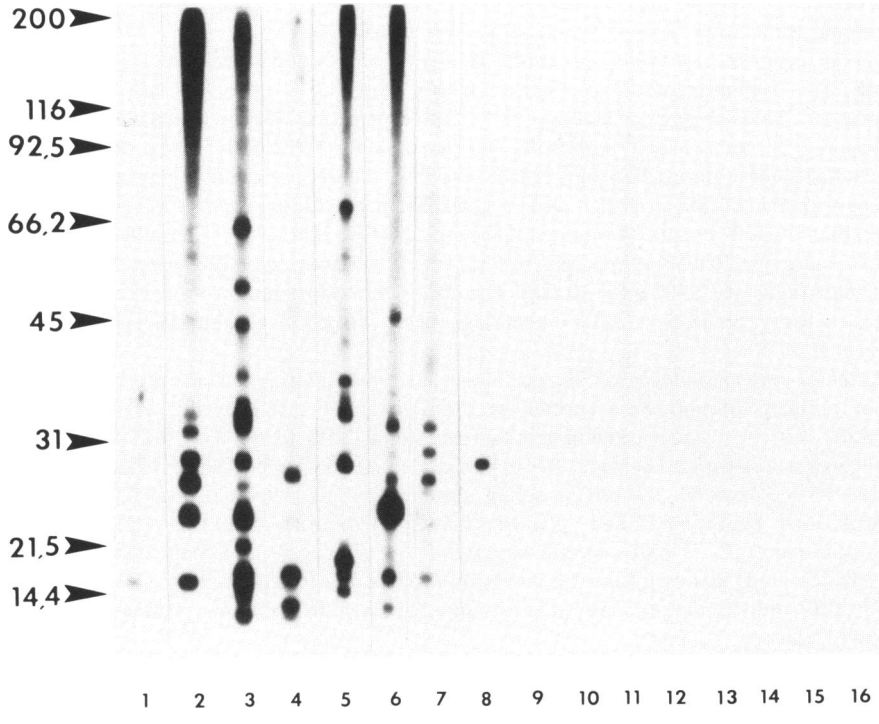

Fig. 1. Autoradiograph of ^{125}I-anti-IgE binding to IgE reacted with soluble *S. aureus* antigens which have been transferred from polyacrylamide to nitrocellulose filters by western blotting. All lanes had 3 days exposure except for lanes 2, 3 and 5, which were exposed overnight. Lanes 1–7 are sera from patients with SAHIGES; lanes 8–12 are sera from *S. aureus* infected control patients without SAHIGES; lanes 13–16 are normal control sera. Numbers correspond to patients as in Table 1. Molecular weight standards are indicated in kD on the left side.

Table 2. IgE antibodies to soluble bacterial antigens

	SAHIGES patients								*S. aureus*-infected patients					Normal controls			
Antigen	1*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
None	119	91	103	105	104	95	113	87	87	80	85	83	85	97	123	95	101
S. aureus	*473*	101	*680*	*790*	*1199*	*539*	*496*	*154*	97	104	102	100	100	111	96	126	112
S. epidermidis	*849*	168	*1708*	*1109*	*1038*	*1472*	*655*	*462*	103	117	103	95	116	119	113	118	109
Strep. group A	172	93	99	116	123	120	113	92	83	85	79	89	87	86	109	113	111
Strep. group B	149	117	87	143	114	147	102	86	96	87	84	83	92	90	102	106	115
H. influenzae b	123	95	90	114	*283*	86	89	92	87	88	77	96	90	98	129	102	101
E. coli	109	97	131	108	109	105	98	86	79	78	87	72	94	85	107	103	93

1* is an earlier serum from patient 1, the other numbers correspond to those of Fig. 1. Results are expressed as counts per minute. Values in italics are more than twice the mean values of the normal controls.

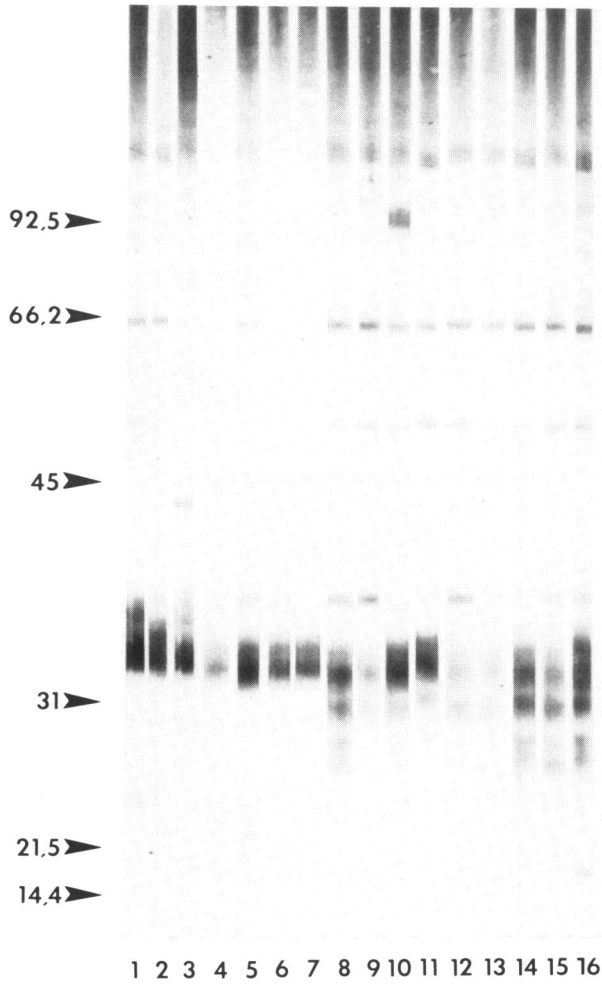

Fig. 2. IgG binding to western blots of soluble *S aureus* antigens detected by anti-human IgG-peroxidase. For identification of individual lanes see legend to Fig. 1.

In a solid phase RIA on nitrocellulose the soluble antigens of *S. epidermidis* show binding of IgE from SAHIGES patients which is equal to or higher than that of similarly prepared *S. aureus* antigens. There is no detectable IgE binding with soluble antigens of *Streptococcus* group A or B, *H. influenzae* or *E. coli* in this assay (Table 2). As in the autoradiograph of Fig. 1, the most recent serum of Patient 1 (lane 1) gives a barely detectable reaction with *S. aureus*; it is noteworthy that this patient has had decreasing concentrations of total serum IgE and anti *S. aureus* PCW IgE over the last few years and has lately developed clinical and serological features of systemic lupus erythematosus (Schopfer *et al.*, 1984). Earlier sera contain IgE to *S. epidermidis* and *S. aureus* (e.g., 1* in Table 2).

IgE binding to staphylococcal antigens is not due to elevated total serum IgE, since Patients 8 and 10 have IgE concentrationtion which are comparable to that of Patient 2 although the IgE binding values are quite different (Table 2). In addition, sera from 16 patients with various allergic diseases and elevated IgE concentrations (>900 IE/ml) have not proven reactive with any of the antigens tested (mean values $\pm$ s.d. for *S. aureus*: 96 ± 16 ct/min and for *S. epidermidis*: 90 ± 23 ct/min).

Staphylococcus aureus infected patients without SAHIGES make an IgG response to soluble *S.*

aureus antigens (Fig. 2), which is generally indistinguishable from that of normal controls. In contrast, seven of seven patients with SAHIGES lack an IgG response to the antigens with a molecular weight below about 31 kD. In addition, six of seven sera from these patients react only weakly or not at all with an antigen of about 53·5 kD. The nitrocellulose strips containing the blotted *S. aureus* antigens do not bind any peroxidase labeled anti IgG thus excluding interference with protein A. ^{125}I-protein A and autoradiography reveal the same binding pattern of selected sera as the peroxidase labelled anti IgG (data not shown).

DISCUSSION

We have detected IgE antibodies to soluble antigens of *S. aureus* in all of seven patients suffering from SAHIGES. This peculiarity of the humoral immune response to *S. aureus* in these patients has not been described so far. In contrast to IgE against PCW (Schopfer, Douglas & Wilkinson, 1980) this IgE response to soluble antigens is not restricted to *S. aureus* but includes similarly prepared antigens from *S. epidermidis*. However, other bacterial antigens tested gave negative results. The production of IgE against many staphylococcal antigens is an abnormal feature of the immune response to staphylococci which is most prominent in patients with SAHIGES. Some *S. aureus* infected control patients and patients with atopic dermatitis may have IgE to staphyloccal antigens in much lower concentrations (Hauser *et al.*, 1985). It is conceivable that these IgE antibodies can mediate an abnormal inflammatory response to staphylococci by interference with neutrophil function (Donabedian & Gallin, 1982; Donabedian & Gallin, 1983; Mawhinney *et al.*, 1980). *Staphylococcus aureus* may thus find suitable conditions for invasion and abscess formation especially at sites of colonization by *S. epidermidis*.

In addition to the abnormal IgE response to staphylococci, our patients with SAHIGES have a qualitatively aberrant IgG response to soluble bacterial antigens, although the difference to the normal IgG response is more subtle than with IgE. The abnormalities essentially consist of a lack of detectable IgG antibodies to certain antigens, which we have not characterized. A protective value of these antibody specificities remains speculative, but futher qualitative analyses of the humoral immune response to *S. aureus* and other pathogens may provide clues to the understanding of apparent pathogen-selective immunodeficiencies for which SAHIGES may be an example.

Even in the absence of any demonstrable pathogenic significance the findings described in this paper further substantiate the fact that SAHIGES patients exhibit a uniquely aberrant antibody profile to many staphylococcal antigens and thereby help to delineate this syndrome from other clinical entities.

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