Table I. Oligonucleotide primers used in PCR test for HPV DNA

Primers	Amplified region	DNA	Map position	Sequence 5′ – 3′	Length of product
MY09	LI33 types	+		CGTCCMARRGGAWACTGATC GCMCAGGGWCATAAYAATGG M = A + C, R = A + G, W = A + T, Y = C + T	450 bp
MY11	HPV	–			
PC03	β-globin	+	62202–62221	ACACAACTGGTTTCACTAGC	
PC04	gene	–	62292–62311	CAACTTCATCCACGTTCACC	110 bp

Table II. Results of analysis for the presence of HPV DNA sequences in DNA isolated in blood and the lesions in individual groups

Studied group	Sex	Number of patients	Verruceous lesions		HPV in lesions		Blood	
			+	–	+	–	HPV+	HPV–
Condyloma acuminata	F	6	2	4	2	–	6	0
	M	6	5	1	5	–	5	1
Common warts	F	2	2	–	2	–	0	2
	M	4	4	–	4	–	0	4
Blood donors	F	6	–	6	–	–	0	6
	M	6	–	6	–	–	0	6

hybridization and polymerase chain reaction. The techniques are particularly useful in the analysis of virus DNA sequences in cells of various tissues. Until now, polymerase chain reaction allowed the presence of HPV to be detected not only in epidermal warts, but also in the unchanged mucous membranes of sexual organs and of the mouth cavity, which proves the potential for latent HPV infections.

On the basis of the obtained results in patients with persisting condyloma acuminata, it has been shown that their blood also contains HPV. This indicates that HPV may become transferred to other sites in the human body; however, the failure to detect HPV DNA sequences in one of the males who is a sexual partner of an HPV infected patient, indicates that the process is controlled by additional factors. Moreover, some forms of sexual relations may cause a predisposition for infection with the virus through various pathways.

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Superantigen Production by *Staphylococcus Aureus* in Atopic Dermatitis: No More Than a Coincidence?

To the Editor:

Atopic dermatitis (AD) has been shown to be frequently colonized by *Staphylococcus aureus* (Leyden *et al*, 1974; Aly *et al*, 1977). *Staphylococcus aureus* could be successfully cultured from lesional and from nonlesional skin of acute and chronic stages of AD (Monti *et al*, 1996). These findings supported the idea of a cause and effect relationship between *S. aureus* and AD (Wakita *et al*, 1995). Further evidence was provided by *S. aureus* being reported to be capable of forming superantigens (McFadden *et al*, 1993; Cooper, 1994; Wakita *et al*, 1995; Strange *et al*, 1996; Yudate *et al*, 1996). Some doubt about the role of *S. aureus* arose when it was demonstrated that corticosteroids (Nilsson *et al*, 1992) were capable of significantly improving AD and decreasing skin colonization by *S. aureus*. Furthermore, only a few investigations were carried out to detect *S. aureus* colonization in the nasal mucosa as a possible reservoir for skin colonization. Therefore, we studied a group of AD patients for the presence of *S. aureus* on the lesional skin as well as for colonization of the vestibulum nasi. The isolates were investigated for superantigen genetic determinants and *in vitro* production. In order to answer the question of whether *S. aureus* from AD patients form a special subgroup of staphylococci, as is the case for *S. aureus* capable of producing toxic shock syndrome toxin 1 (TSST-1) or exfoliative toxins (Johnson *et al*, 1991), we subjected these strains to molecular population analysis and compared them with *S. aureus* from nasal colonization of healthy humans. Colonization of skin and anterior nares by *S. aureus* was studied in 35 patients, aged 3 mo to 53 y, with different stages of AD, and in a group of healthy students. Sixty students were investigated concerning nasal colonization, and 20 of 60 were additionally checked for skin colonization. Twenty-two of 35 patients showed acute exacerbation of AD at the time of admission. They had not received antibiotics during the last 6 mo and were examined either as outpatients or at the first day of hospitalization. Nose swabs were taken from one

Manuscript received November 15, 1997; revised December 23, 1997; accepted for publication January 14, 1998.

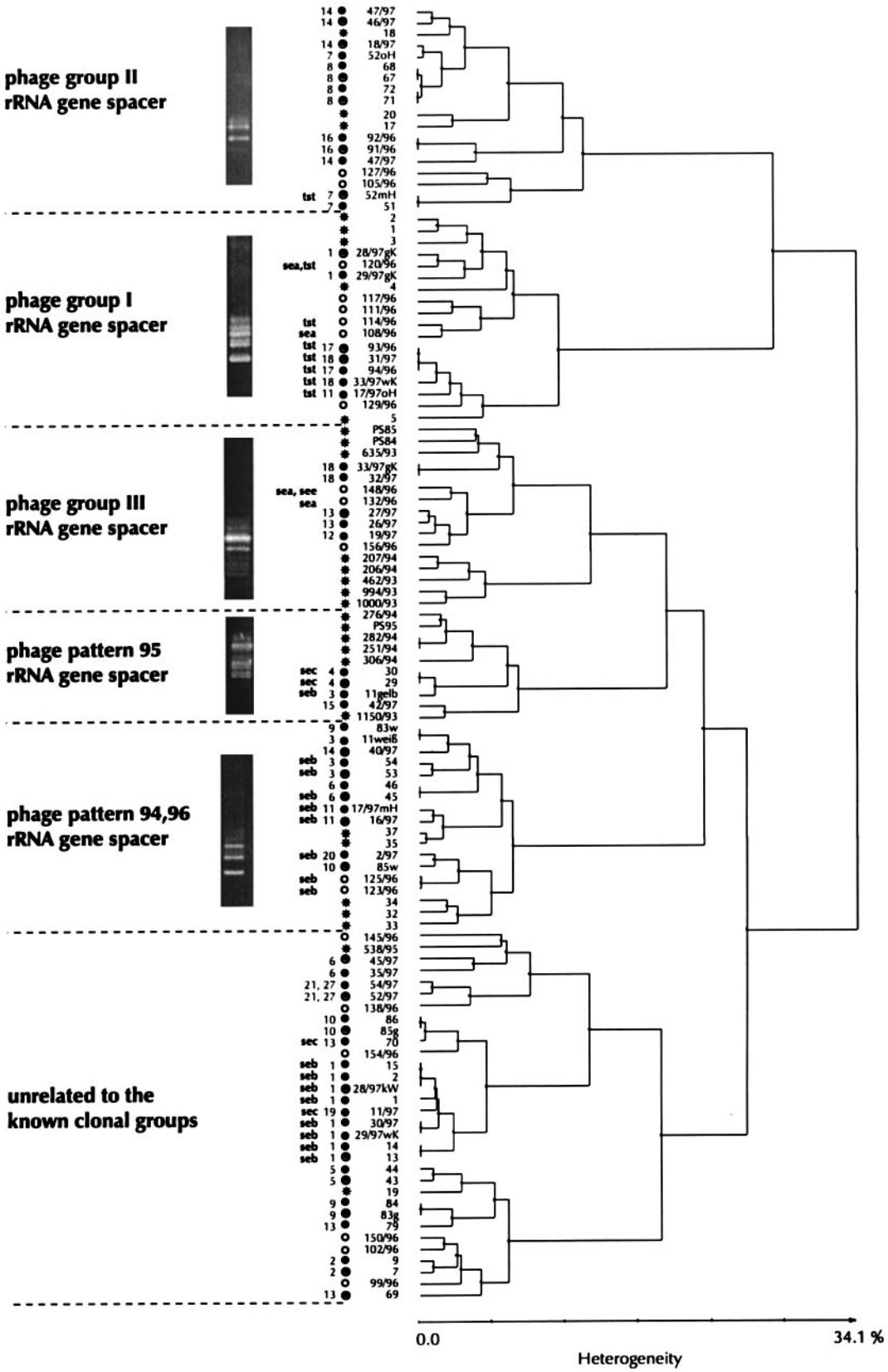

Figure 1. *SmaI* macrorestriction patterns of *S. aureus* from patients with AD and from a control group. ●, Strains from affected skin in AD, strains from nasal swabs of AD patients; arabic numbers designate individual patients; ⊙, strains from nasal swabs of healthy carriers; ○, reference strains of the major clonal groups of the species *S. aureus*.

anterior nare with sterile cotton tips. Skin swabs were obtained from lesional skin of one volar forearm or further lesional areas and applied on blood agar plates. The cultures were grown overnight (37°C in 5% CO_2). Colonies of *S. aureus* were subcultured on sheep blood agar plates for identification by testing coagulase activity and crystal violet type. Antibiotic susceptibility was assessed

by microbroth dilution to determine minimal inhibitory concentration according to the National Committee for Clinical Laboratory Standards (1995). *Staphylococcus aureus* strains were phage typed using the International Phage Set and additional phages. *Staphylococcus aureus* strains were assayed for production of staphylococcal enterotoxins (SEA-D) and TSST-1 by the Latex agglutination test from Oxoid. Capacity for superantigen formation was assessed by polymerase chain reaction for toxin genes as described previously (Johnson *et al*, 1991). Molecular population analysis was performed by means of Serratia marcescens (*SmaI*) macrorestriction analysis and r-RNA gene spacer patterns (Cuny and Witte, 1996). *SmaI* macrorestriction patterns were allotted to those of *S. aureus* representative for clonal groups of these strains and stored in a databank system. Agarose gels were image processed, and the molecular masses of fragments were stored in the databank and analyzed for similarity as previously described (Cuny and Witte, 1996). In 22 of 35 patients (62.9%), *S. aureus* was isolated from one or more of the selected skin areas. Twenty of the 22 *S. aureus* positive AD patients were also nasal carriers. Eighteen patients revealed the same strain in skin colonization and nasal mucosa. In four of 13 AD patients with negative skin but with positive nose swabs, one strain produced staphylococcal enterotoxin C. Eighteen of 60 (30%) of the healthy volunteers from the control group revealed *S. aureus* colonization in the nasal cavity. Only 10 of 22 strains (45%) of *S. aureus* isolated from AD patients were capable of producing superantigens (enterotoxins A, B, C, TSST-1), as evidenced by detection of the extracellular product and polymerase chain reaction for the corresponding genetic determinants. This frequency corresponds with the results for superantigen production of *S. aureus* strains isolated from eight of 18 healthy carriers (44%). No predominance of a certain enterotoxin or a clonal group of *S. aureus* was found. There was no correlation between *S. aureus* strains capable of superantigen production and total IgE or eosinophilic cationic protein plasma levels. Molecular typing of *S. aureus* from AD patients by means of *SmaI* macrorestriction and r-RNA gene spacer patterns revealed the same population structure as demonstrated for *S. aureus* from nasal colonization. Data are given in the similarity dendrogram (**Fig 1**). Staphylococcal colonization is regarded as a constant phenomenon in AD patients and is reported to be independent of age and stages of skin inflammation. Previous studies revealed no difference between children and adults concerning nasal colonization with *S. aureus* and superantigen formation (Mochmann *et al*, 1981; Richter *et al*, 1981). Hoeger *et al* (1992) found that the *S. aureus* colonization rate observed in children with AD did not differ from that reported for adult AD patients. Ogawa *et al* (1994), who report a comparative study of staphylococcal flora on the skin surface of AD patients and healthy subjects, did not find sex dependent differences in their patients. The *S. aureus* carriage rate of 62.9% in our AD study group ranges at the lower limit of those described by others (Bahkdi and Tranum-Jensen, 1991; McFadden *et al*, 1993; Monti *et al*, 1996). In 81.9% of our AD patients identical *S. aureus* strains were isolated from nose and skin swabs, as described previously (Goodyear *et al*, 1993). Several studies found a relatively high rate of colonization with toxigenic *S. aureus* strains in AD patients (64–100%) (Bahkdi and Tranum-Jensen, 1991; Leung *et al*, 1993; McFadden *et al*, 1993), so that a causative role of superantigens in AD was discussed. In our controlled study only 45% of strains from AD patients were capable of producing superantigens (SEB, SEC, TSST-1) as evidenced by detection of the extracellular product and polymerase chain reaction for the corresponding genetic determinants; however, this frequency does not exceed the number of *S. aureus* strains isolated from healthy carriers (44%) capable of superantigen production. No predominance of a certain toxin was found in contrast to other authors, who reported SEA (Hoeger *et al*, 1992) or TSST-1 (Jacobson *et al*, 1989) to be the most commonly identified toxin. *Staphylococcus aureus* colonization was proposed to be a stimulant for IgE production from *in vitro* experiments (Neuber and König, 1992), especially the toxigenic ones. This could not be confirmed in our *in vivo* study.

The prevalence of particular *S. aureus* strains in AD has been suggested, but phage typing results did not support the existence of any predominant biotype (Hoeger *et al*, 1992). This is in accordance with our results. Molecular typing of *S. aureus* strains from AD patients by means of *SmaI* macrorestriction and r-RNA gene spacer patterns revealed the same population structure as *S. aureus* from nasal colonization in normals. This indicates that the occurrence of *S. aureus* in AD is not associated with a particular group of strains. Our results do not support the hypothesis that skin colonization with *S. aureus* and especially superantigen formation are an essential prerequisite in the pathogenesis of AD. This study, however, does not exclude a possible role of *S. aureus* superantigens in the acceleration of AD individual clinical course in the case of skin colonization with superantigen producing *S. aureus*.

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