

Bacteria and endotoxin induce release of basophil histamine in patients with atopic dermatitis

In vitro experiments with *S. aureus*, teichoic acid, *E. coli* and *E. coli* LPS

To the Editor,
Patients with atopic dermatitis (AD) contain huge amounts of *Staphylococcus aureus* on the skin, especially at the lesional sites (7). The bacteria may be dispersed and lead to *S. aureus*-specific immune response in AD patients, since increased serum IgE has been found to whole cells, cell wall and soluble antigens of *S. aureus* (1, 5, 6, 8). Increased intestinal permeability has been discussed in AD and suggested by the finding of increased urinary excretion of perorally administered polyethylene glycol (9) and lactulose/mannitol (15), whereas ⁵¹Cr-labelled EDTA absorption test showed no difference from normal individuals (2). With increased permeability this might lead to the entrance of bacteria and endotoxins.

We have examined the possibility of allergic type I reactions directed against *S. aureus* and its cell wall component, teichoic acid, and, furthermore, against *Escherichia coli* bacteria and their endotoxins (LPS), by the basophil histamine release technique.

Blood was obtained from 18 AD patients comprising nine children (2 to 16 years) and nine adults (19 to 48 years) and from age-matched controls (healthy volunteers). Leukocytes containing approximately 2% basophils were isolated by the Ficoll-Hypaque gradient method, washed twice, and suspended in glucose-free Tris-AMC (albumin, magnesium, calcium) buffer (13). The leukocyte suspension was incubated for 40 min at 37°C with whole bacteria of the *S. aureus* strain Wood 46 (protein A deficient) (4) and for *E. coli* (11), and also with *E. coli* LPS preparations of the Boivin type (11) and teichoic acid isolated from the bacterial cell wall of Wood 46, as previously described (3, 12). Bacteria and teichoic acid were used in

final concentrations of 1.25, 2.5, 5 and 10 mg/ml and LPS in 0.1, 10 and 100 µg/ml. The release of histamine from the basophils was determined spectrofluorometrically (13). A positive response was defined as a release of more than 10% of the total histamine content in the sample, since the spontaneous release of histamine amounted to up to 10%. To examine whether the histamine release was caused by an allergic (IgE-dependent) or an immunoglobulin-independent mechanism, cell-bound IgE was removed from the basophils by exposure of the cell suspension to pH 3.8 for 5 min at 4°C (14) before challenge of the cells with bacteria, LPS or teichoic acid.

If histamine release was obtained from intact cells armed with specific IgE but not from the cells stripped of IgE, the mechanism would be IgE-dependent (10). If both the intact and the stripped cells caused a similar release of histamine, an immunoglobulin-independent mechanism would be suggested (10).

Of the 18 AD patients eight (45%) responded

Table 1

Allergic histamine release caused by bacteria and their products in leukocyte suspensions from AD patients and normal age-matched individuals

No. of subjects	Per cent responders to			
	<i>S. aureus</i>	<i>S. aureus</i> Teichoic acid	<i>E. coli</i> LPS	<i>E. coli</i>
AD pts.				
18	45	56	56	50
Controls				
13	15	15	0	0

Note that no difference was found in % responders between children and adults.

to *S. aureus* with an IgE-dependent mechanism, whereas this was only found in two of 13 controls (15%) (Table 1). Cells from the remaining individuals in both groups released histamine by an immunoglobulin-independent mechanism. The response to *S. aureus* was well correlated to that observed to teichoic acid, since exactly the same persons in the control group and in the group of AD patients responded to teichoic acid with allergic and immunoglobulin-independent histamine release, respectively. Only two of the 18 AD patients showed a dissociation, since the histamine release induced by *S. aureus* was due to an immunoglobulin-independent mechanism, whereas that induced by teichoic acid was due to an IgE-dependent mechanism.

When cells from the control group were challenged with *E. coli* LPS, no histamine release was obtained. This is in accordance with our earlier findings with endotoxins from *E. coli* and *Salmonella* bacteria (11). However, *E. coli* LPS caused histamine release in 10 (56%) of 18 AD patients by an IgE-dependent mechanism (Table 1), while no histamine release was seen in the remaining patients. The endotoxin response was well correlated to that observed to *E. coli* whole cells. Thus, AD patients showing IgE-dependent histamine release induced by the endotoxin also demonstrated this response by challenge with *E. coli* bacteria, with the exception of one person who did not respond to the bacteria. Furthermore, in analogy with the endotoxin, *E. coli* bacteria caused no IgE-dependent histamine release in the control group.

On the basis of our findings it is tempting to speculate that in AD patients the higher incidence of IgE-dependent reactions to whole bacteria of *S. aureus* and its cell wall component, teichoic acid, might be due to a sensitization to the bacterium and its component by penetration through the lesional sites of the skin or through the mucosal membranes of the upper respiratory tract. It is remarkable that endotoxin, which does not induce the release of histamine either in normal individuals or in grass pollen-allergic patients (11), released histamine in AD patients by an IgE-dependent mechanism, and

this was also found for *E. coli* bacteria. The endotoxin response was also demonstrated in some AD patients without food allergy. If increased intestinal permeability is present in AD patients, this would explain the response to *E. coli* bacteria and their endotoxins. In summary, our findings suggest the possibility of allergic type I reactions in atopic dermatitis directed against *S. aureus* and its teichoic acid component and, furthermore, against *E. coli* bacteria and their endotoxins.

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