

# Increased plasma tumour necrosis factor- $\alpha$ concentration in atopic dermatitis

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## Abstract

**Plasma tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) was measured in 15 children with atopic dermatitis, 13 children with bronchial asthma, and 11 healthy controls. Plasma TNF- $\alpha$  concentration was increased in atopic dermatitis and the magnitude of the increase was correlated with the severity of the dermatitis but TNF- $\alpha$  concentration was not increased in bronchial asthma. A significant correlation was found between plasma TNF- $\alpha$  and plasma histamine concentrations in atopic dermatitis. The data suggest that the overproduction of TNF- $\alpha$  is associated with increased plasma histamine concentration, and might play a part in the pathophysiological mechanism of atopic dermatitis.**

Atopic dermatitis is a chronic, pruritic, and inflammatory skin disease associated with several immunological abnormalities. These include an increase in serum IgE, decreased number of CD8+ T cells, reduced lymphocyte blastogenesis to mitogens, low incidence of positive skin tests, and depressed granulocyte and monocyte chemotaxis.<sup>1</sup>

Recent reports have suggested that the alteration of cytokine production may play a part in the pathogenesis of atopic dermatitis.<sup>2,3</sup> In the present study, plasma tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentrations in atopic dermatitis and in bronchial asthma were compared with those in healthy controls. In addition, the association between plasma TNF- $\alpha$  and plasma histamine was investigated, as plasma histamine concentration has been reported to be increased in atopic dermatitis.<sup>4,5</sup>

## Subjects and methods

### SUBJECTS

Children visiting the paediatric department at Sumitomo Hospital were enrolled in this study. Fifteen patients with atopic dermatitis but without bronchial asthma (seven boys and eight girls) with a mean age of 3.3 years (range 1-15 years) were divided into three subgroups according to the severity of dermatitis (mild, moderate, or severe).<sup>6</sup> Thirteen patients with bronchial dermatitis but without atopic dermatitis (seven boys and six girls) with a mean age of 5.2 years (range 2-14 years) and 11 healthy controls (five boys and six girls) with a mean age of 4.6 years (range 3-10 years) were also studied. None of the patients was febrile, currently asthmatic, or receiving systemic steroids.

### PLASMA AND SERUM

Fresh plasma was obtained from all subjects for the assay of histamine and TNF- $\alpha$ , and from patient's serum for IgE concentrations. Blood samples for plasma were collected in tubes containing EDTA, placed on ice immediately, and stored at -20°C until required.

### TNF- $\alpha$ assay

Concentrations of TNF- $\alpha$  were determined using an immunoradiometric assay with a sensitivity of 1 pg/ml (IRE-Medgenix). Polypropylene tubes were coated with a combination of monoclonal antibodies to recombinant human TNF- $\alpha$ , which recognise distinct epitopes of TNF- $\alpha$ . Samples were incubated overnight in these tubes at room temperature with a mixture of <sup>125</sup>I-labelled anti-TNF- $\alpha$  antibody. After decantation, the bound fraction was counted with a gamma counter, and the concentration of TNF- $\alpha$  was expressed in terms of pg/ml with reference to a standard binding curve of recombinant human TNF- $\alpha$ .

### HISTAMINE ASSAY

Concentrations of plasma histamine were determined using a new radioimmunoassay developed by Immunotech. Samples were placed into acylation tubes, to which acylation buffer was added, followed by centrifugation. <sup>125</sup>I-labelled acylated histamine was then added, after which the materials were centrifuged. Solutions were transferred to monoclonal antibody coated tubes, then incubated at 4°C overnight. After decantation, the bound fraction was counted with a gamma counter, and the concentration of histamine was expressed as nmol/l with reference to a standard binding curve of acylated histamine.

### IgE QUANTIFICATION

Serum IgE concentrations were determined by solid phase radioimmunoassay (Phadebas IgE Prist, Pharmacia).

### STATISTICAL ANALYSIS

All results are reported as means (SD). Student's *t* test was used to compare mean values.

## Results

### TNF- $\alpha$ CONCENTRATIONS IN PLASMA

As shown in fig 1, TNF- $\alpha$  was undetectable (<15 pg/ml) in plasma from most of the healthy controls, and TNF- $\alpha$  concentration was only

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slightly increased in plasma from bronchial asthma patients. In contrast, patients with atopic dermatitis showed increased TNF- $\alpha$  concentrations in plasma, and the magnitude of increase was correlated with the severity of the dermatitis.

#### HISTAMINE CONCENTRATIONS IN PLASMA

Figure 2 shows that plasma histamine concentration was higher in patients with atopic

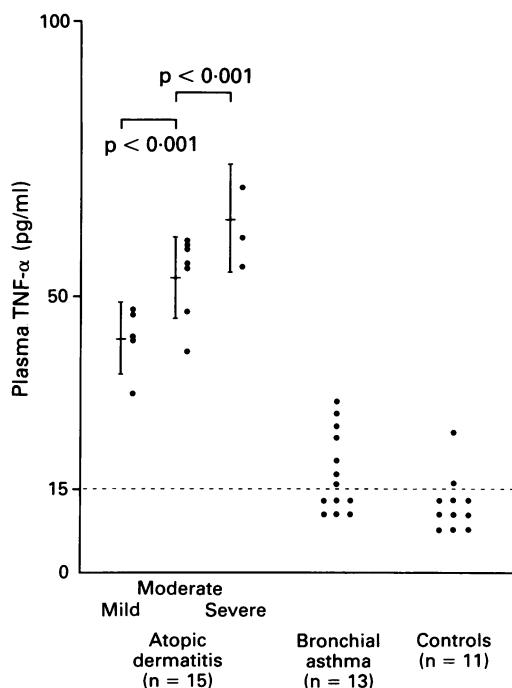


Figure 1 Plasma TNF- $\alpha$  concentrations in children with atopic dermatitis and with bronchial asthma, and in healthy controls. Dotted line indicates lowest detectable limit for TNF- $\alpha$ .

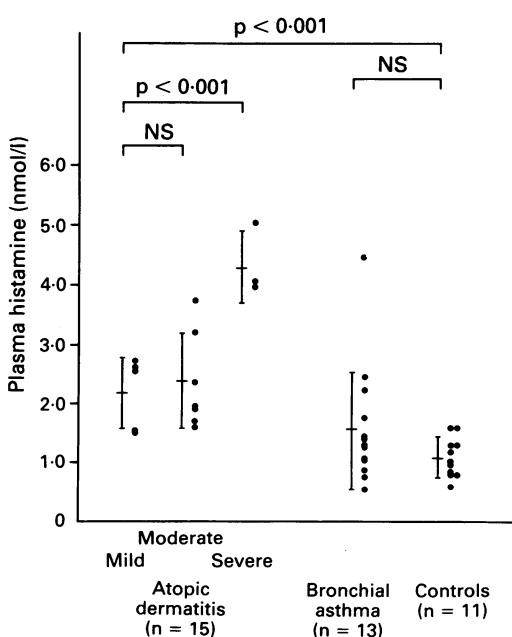


Figure 2 Plasma histamine concentrations in children with atopic dermatitis and with bronchial asthma, and in healthy controls.

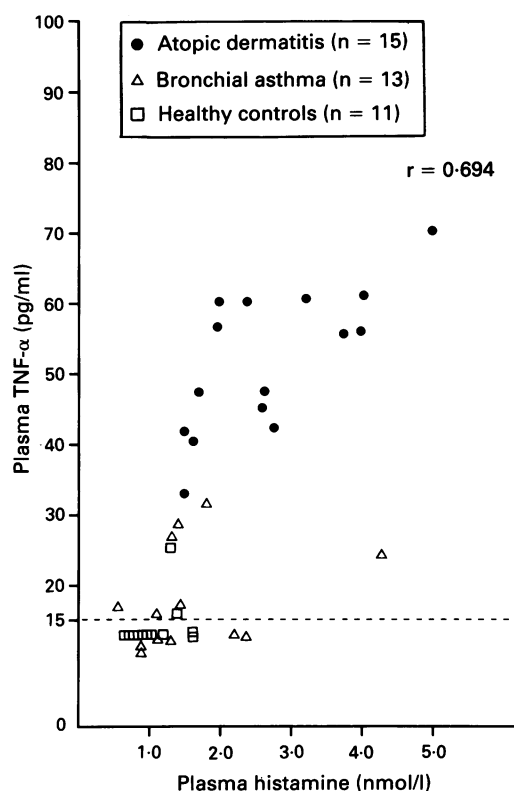


Figure 3 Relationship between plasma TNF- $\alpha$  and plasma histamine in children with atopic dermatitis and with bronchial asthma, and in healthy controls. Dotted line indicates lowest detectable limit for TNF- $\alpha$ .

dermatitis (2.70 (1.09) nmol/l) than that in healthy controls (1.09 (0.35) nmol/l;  $p < 0.001$ ), and that the magnitude of increase was correlated with the severity of the atopic dermatitis.

**RELATIONSHIP BETWEEN TNF- $\alpha$  AND HISTAMINE**  
As shown in fig 3, there was a significant correlation between concentrations of plasma TNF- $\alpha$  and plasma histamine in patients with atopic dermatitis ( $r = 0.694$ ;  $p < 0.01$ ), while there was no significant correlation between concentrations of plasma TNF- $\alpha$  and serum IgE in these patients ( $r = 0.079$ ).

#### Discussion

In the present study, we found that plasma TNF- $\alpha$  was increased in atopic dermatitis, and that the magnitude of increase was correlated with the severity of the dermatitis. The findings also show that in these patients there is an association between concentrations of plasma TNF- $\alpha$  and plasma histamine, but no relationship between concentration of plasma TNF- $\alpha$  and serum IgE.

TNF- $\alpha$ , a cytokine secreted by activated monocytes and macrophages, has been shown to produce a wide variety of biological effects in humans.<sup>7-9</sup> Some of these effects are associated with infection,<sup>10</sup> inflammation,<sup>11 12</sup> and immunoregulation.<sup>13</sup> According to a recent report, TNF- $\alpha$  is produced in keratinocytes,<sup>14</sup> and it may play a regulatory part in dermal-

epidermal interactions during wound healing, inflammation, and epidermal growth and differentiation.<sup>15</sup> After intravenous administration of TNF- $\alpha$ , a highly significant amount (30%) of it localises in the epidermis.<sup>16</sup> Along with granulocyte/macrophage colony stimulating factor and interleukin-1, TNF- $\alpha$  regulates Langerhans cell viability and differentiation,<sup>17</sup> and it also activates inflammatory cells such as neutrophils, eosinophils, macrophages, and fibroblasts.<sup>18</sup>

It is not known whether the increase in plasma TNF- $\alpha$  in our study was due to production by monocytes in peripheral blood or by macrophages and keratinocytes in the epidermis. The increased plasma TNF- $\alpha$  concentration suggests that the epidermal level is high, a condition which may increase the severity of atopic dermatitis. Although we could not determine the mechanism by which increased TNF- $\alpha$  concentration increases the severity of atopic dermatitis, it might be associated with plasma histamine, but is not associated with serum IgE.

Atopic dermatitis is a multifactorial disease, and hereditary, environmental, and immunological factors are all involved in its pathogenesis. The findings of the present study suggest that TNF- $\alpha$  may be related to the pathophysiological mechanism of atopic dermatitis.

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