

allergic bronchopulmonary fungal disease. Asthmatic patients are susceptible to allergic fungal reactions, and eosinophilia is one of the characteristic features of this syndrome. Although *Aspergillus* species are the main causative organisms, it is now recognized that many other fungi can be associated with this syndrome (1).

A. pullulans is a yeast-like fungus found in leaves, soil, and water. Although widely prevalent in the world, the level of its spores in air is low. It is a rare human pathogen, and it has been found to be the responsible allergen in few outbreaks of allergic pulmonary reactions, mostly due to contamination of heating, cooling, and ventilation units by this fungus (2).

Thromboembolism is a well-known manifestation of the idiopathic hypereosinophilic syndrome (IHS). Cardiac involvement in IHS is the main cause of death in this syndrome, and mural thrombosis is characteristic of IHS. Other thromboembolic events have been described in this syndrome, affecting the brain, lungs, retina, and intra-abdominal veins (such as the hepatic vein) (3).

Thromboembolic events were also found in transient, unsustained conditions of eosinophilia such as allergic reactions to food, drugs, or aeroallergens (4). The association between eosinophilia and thrombophilia was first suggested by Fauci et al. in 1982 (3). The proposed pathogenesis was the direct cytotoxic effect of the eosinophils on the endothelium or endocardium. More recent evidence suggests that eosinophil-derived mediators can modulate the coagulation cascade to promote thrombosis (5). It was found that eosinophil-specific granule proteins, particularly major basic protein (MBP), inhibit the anticoagulant effect of thrombomodulin (6, 7).

In summary, the patient presented here had severe eosinophilia most probably caused by an allergic reaction to *A.*

pullulans. This severe reaction, accompanied by marked eosinophilia, caused a thrombotic diathesis and widespread thromboembolism. This case illustrates the association of allergic conditions in general and eosinophilia in particular with thromboembolism. Hence one should bear in mind that even moderate eosinophilia may lead to thrombophilia resulting in thromboembolic manifestations.

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CD124 on monocytes in grass-pollen allergy

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● ALLERGIC individuals display a still unrecognized failure of IFN- γ production and/or excessive production of IL-4 that may affect the Th1-Th2 balance (1). Decreased production of IFN- γ by allergic PBMC could depend on defective

production of other regulatory cytokines, such as monocyte (M ϕ)-derived

IL-4 receptor overexpression could explain IFN- γ decrease.

IL-12, a strong stimulator of IFN- γ production. Indeed, there have been reports that the M ϕ of allergic patients produced a smaller amount of IL-12 (2). Recently, IL-4 has been found to suppress IL-12 production (3). Therefore, this study aimed to compare the M ϕ response to IL-4 of allergic and healthy individuals *in vitro*.

The study involved monoallergic grass-pollen-sensitive patients ($n=16$) with positive skin tests, total IgE of >400 IU/ml, and moderate seasonal allergic symptoms. The patients were examined in a grass-

pollen-free period, at least 2 months before the expected natural allergen exposure.

Neither systemic corticosteroids nor nonsteroid anti-inflammatory agents were given to patients at least 1 month before and during the study. The control group consisted of healthy volunteers ($n=10$) with no history of allergic reactions and total IgE of <50 IU/ml.

PBMC (2×10^6 cells/ml) obtained by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation of heparinized blood were cultured with $40 \mu\text{g/ml}$ PHA (Wellcome, Dartford, UK). The adherent fraction of PBMC, corresponding to the Mø population, was cultured with PHA, huIL-4 (10 ng/ml ; Biosource, Camarillo, CA, USA), or a combination of both. After 48-h culture, IL-4 and IFN- γ concentration in PBMC-derived supernatants and IL-12 in Mø fraction supernatants were measured by Predicta-ELISA (Genzyme, Boston, MA, USA).

For the FACS analysis, PBMC were incubated with anti-CD124 monoclonal antibodies (mAbs) or isotype control (Genzyme, Cambridge, MA, USA). Samples were then incubated with FITC-conjugated anti-immunoglobulin F(ab')₂ (DAKO, Glostrup, Denmark) followed by incubation with R-phycoerythrin-conjugated anti-CD14 mAb, or with isotype control (both from DAKO). The analysis was done by FACSCalibur with CellQuest software (Becton-Dickinson, San Diego, CA, USA). The percentage of CD14⁺ CD124⁺ and CD14⁺ CD124⁺ cells and mean fluorescence intensity (MFI), corresponding to CD124 expression, were estimated.

No significant difference between allergic and control patients in spontaneous IL-4, IFN- γ , or IL-12 production was observed. Moreover, after PHA stimulation of PBMC, IL-4 concentration in allergic and controls did not differ. We found a significantly lower IFN- γ concentration in supernatants from PHA-stimulated allergic PBMC than

in the control (650 ± 175 vs 1720 ± 250 pg/ml, respectively, at $P<0.002$).

Although the IL-12 level in supernatants from PHA-stimulated allergic Mø was lower than in controls, the difference was not significant. The analysis of huIL-4 influence on Mø revealed a strong suppressive activity of this cytokine on PHA-induced IL-12 production. IL-4 suppressed IL-12 production in allergic Mø more effectively than in the control; however, the observed difference was not significant ($P=0.07$).

FACS analysis of PBMC revealed no difference in the percentage of CD124⁺ cells between allergic and healthy individuals ($58 \pm 8\%$ and $62 \pm 9.5\%$, respectively). Approximately 40–45% of CD124⁺ cells coexpressed the CD14 molecule and displayed typical Mø structure, as found by immunostaining and microscope examination. On the other hand, all CD14⁺ PBMC coexpressed CD124. No statistically significant difference in percentage of CD14⁺ CD124⁺ or CD14⁺ CD124⁺ PBMC between allergics and controls was observed. The MFI, corresponding to CD124 expression on allergic Mø, was significantly higher than in the control group (483.2 ± 50 vs 696.2 ± 75 ; respectively, at $P<0.04$).

Constitutive expression of IL-4R has been detected in many types of cells. The increase of CD124 (specific IL-4R α -subunit) has been shown in bronchial and nasal mucosa-biopsy specimens of allergic individuals, but the cells responsible for this effect were not certainly identified (4). As shown in our study, the Mø of allergic patients expressed CD124 in higher amounts than the Mø of healthy controls. Thus, IL-4 could suppress IL-12 production by allergic Mø even if it was present in a similar concentration, as in unaffected individuals. Presumably, IL-4-mediated inhibition of IL-12 production by Mø could result in decreased IFN- γ expression in allergy. Thus, our observations extend previous reports suggesting the importance of IL-4R increase

in allergy. However, the cause of CD124 overexpression on allergic Mø remains to be elucidated.

CD124 overexpression on monocytes could explain the IFN- γ decrease in allergy.

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