

**TABLE I.** Reasons for immunotherapy withdrawals in the 3 groups of subjects who underwent immunotherapy administration by means of SCIT, SLIT, or LNIT

|                 | SCIT  | SLIT  | LNIT  |
|-----------------|-------|-------|-------|
| Ineffective     | 13.0% | 24.9% | 18.3% |
| Expensive       | 39.6% | 36.4% | 13.3% |
| Unpleasant      | 8.7%  | 5.8%  | 56.6% |
| Time consuming  | 24.2% | 17.9% | 3.5%  |
| Family problems | 14.5% | 15.0% | 8.3%  |

was stopping immunotherapy without the authorization of the prescribing physician.

The patients who withdrew immunotherapy before the planned period of 3 years from the start of immunotherapy were considered noncompliant. In the whole study population, 440 (15.9%) of 2774 patients were considered noncompliant.

In terms of compliance, no significant differences were found between the patients followed in a hospital setting (87.2%) and those followed in a private medical office setting (81.7%).

The percentage of noncompliant individuals was significantly higher in group C (LNIT, 73.2%) with respect to both group B (SLIT, 21.5%;  $\chi^2 = 10.28$ ,  $P < .005$ ) and group A (SCIT, 10.9%;  $\chi^2 = 25.92$ ,  $P < .0005$ ). The percentage of noncompliant patients in group A was also significantly lower than in group B ( $\chi^2 = 17.1$ ,  $P < .0005$ ).

In groups A and B only 6.4% of patients withdrew immunotherapy very early (ie, during the first 12 months), whereas in group C the prevalence of patients who withdrew immunotherapy throughout the first 12 months was significantly higher (43.9% vs 6.4,  $P < .0005$ ).

Times at treatment withdrawal in the 3 groups during the overall 3-year period are analytically illustrated in Fig 1. Reasons for immunotherapy withdrawal in the 3 groups, as determined from specific interviews with the patients' parents, are summarized in Table I.

The most common cause for treatment withdrawal both in groups A and B was the cost. For group B, another common reason for stopping immunotherapy was represented by an insufficient effectiveness. In Group C, the preponderant reason was represented by the discomfort for the repeated nasal reactions, which explains the very early interruption of immunotherapy in 43.9% of the patients belonging to this group. Therefore the nasal route of administration, even though scientifically demonstrated as effective, does not represent in practice an advisable option for immunotherapy.

In summary, SCIT, although expensive and time consuming, is considered to date the most suitable immunotherapy form and route of administration in children and adolescents. SLIT, however, is well accepted by more than three quarters of the patients.

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## Plasma mannan-binding lectin levels and activity are increased in allergic patients

To the Editor:

The present report documents the presence of increased plasma levels of functionally active mannan-binding lectin (MBL) and their association with clinical markers in patients with respiratory allergic diseases. Respiratory allergic disorders, such as asthma and allergic rhinitis, result from a systemic inflammatory reaction triggered by allergens in genetically susceptible hosts. In addition to IgE- and T<sub>H</sub>2-mediated events, a pathogenic role of complement pathways and their activation products C3a and C5a has been implicated in asthma and allergic inflammation.<sup>1</sup> Earlier studies showed the activation of

classical and alternative pathways of complement during bronchial asthma.<sup>2</sup>

Another important complement pathway is the lectin complement cascade, which is initiated by a multifunctional C-type serum lectin, MBL.<sup>3</sup> MBL is an important effector molecule of innate immunity that binds allergens and modulates the levels of proinflammatory cytokines.<sup>3</sup> Henceforth, we determined the MBL level and its complement activity in allergic patients and studied its correlation with peripheral blood eosinophilia and percent predicted FEV<sub>1</sub>, 2 of the commonly used markers for bronchial inflammation in allergic airway diseases.

A case-control study was conducted after obtaining approval of the human ethics committee of Vallabhbhai Patel Chest Institute and the Institute of Genomics and Integrative Biology.

Blood samples were collected from Indian individuals (n = 95), and the sample included allergic patients (n = 55) of 2 categories, patients with bronchial asthma with allergic rhinitis (n = 44) and patients with allergic bronchopulmonary aspergillosis (ABPA; n = 11), and unrelated, age-matched healthy control subjects of similar ethnicity without any history of bronchial asthma, allergic rhinitis, ABPA, or other respiratory symptoms (n = 40). The age of the patients ranged from 12 to 42 years, and that of the control subjects ranged from 15 to 48 years. The asthmatic patients were included if they were 12 years of age or older and had never smoked and if asthma was confirmed by demonstrating a reversibility of at least 12% and an increase of 200 mL in FEV<sub>1</sub> after inhalation of 200 µg of salbutamol from a metered-dose inhaler. All patients with bronchial asthma had a positive skin prick test response against at least one of the tested local allergens. Patients showing the presence of central bronchiectasis, *Aspergillus fumigatus*-specific antibodies, immediate cutaneous reactivity to *Aspergillus* species mixes or *A fumigatus*, and increased total serum IgE levels (>1000 ng/mL) were included as patients with ABPA in the study. None of the control subjects had a positive skin test response to any of the allergens tested.

The range of peripheral blood eosinophilia was 2% to 12% in patients with bronchial asthma with allergic rhinitis, 2% to 20% in patients with ABPA, and 0% to 5% in control subjects. The range of percent predicted FEV<sub>1</sub> was 33% to 86% in patients with bronchial asthma with allergic rhinitis, 24% to 86% in patients with ABPA, and 87% to 98% in the control subjects.

Informed written consent was obtained from all participants. The results of all the experiments are expressed as mean (SD) units. Statistical significance of the data was determined by using the paired Student *t* test. A *P* value of less than .05 was considered significant.

MBL levels in the plasma samples were estimated as previously described.<sup>4</sup> For MBL activity, we measured the deposition of C4b by MBL, the first product of MBL-induced lectin pathway of complement, on mannan-coated microtiter plates by using rabbit anti-C4 polyclonal antibody and peroxidase-conjugated goat anti-rabbit polyclonal antibody.<sup>5</sup> The mean MBL level (3.28 µg/mL [SD,

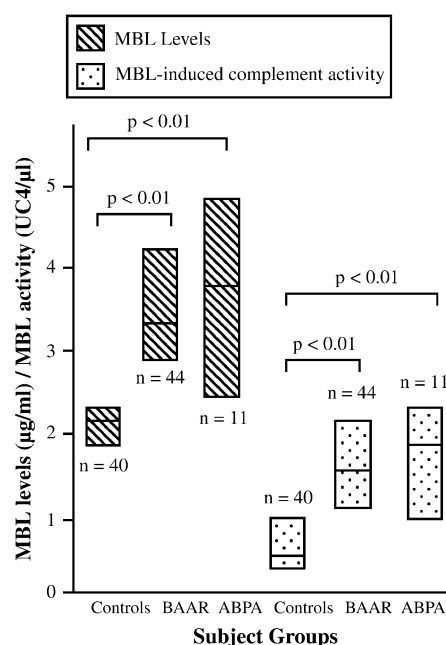


**FIG 1.** Box diagrams of MBL levels (in micrograms per milliliter) and MBL-induced complement activity (in UC4 per microliter) in patients with bronchial asthma with allergic rhinitis (BAAR), patients with ABPA, and control subjects, with mean values indicated by lines and plotted with the online statistical package PAST, version 1.34 (Hammer and Harper, Oslo, Norway).

0.86 µg/mL]) and the mean MBL-induced complement activity (1.46 Units of C4b deposited [UC4]/µL [SD, 0.74 UC4/µL]) in the allergic patients was significantly higher than the mean MBL level (2.08 µg/mL [SD, 0.505 µg/mL]) and the mean MBL-induced complement activity (0.592 UC4/µL [SD, 0.364 UC4/µL]) of the control subjects (*P* < .01, Fig 1). It is noteworthy to mention here that high levels of C-reactive protein, an acute-phase serum protein that activates the classical complement pathway, have also been positively associated with atopic diseases.<sup>6</sup>

MBL levels and complement activity showed a significant positive correlation with the peripheral blood eosinophilia of patients with ABPA (*r* = 0.83 and *r* = 0.76, respectively) and patients with bronchial asthma with allergic rhinitis (*r* = 0.67 and *r* = 0.68, respectively; see Fig E1 in the Online Repository in the online version of this article at [www.jacionline.org](http://www.jacionline.org)). As expected, a negative correlation of lung performance (percent predicted FEV<sub>1</sub>) was observed with MBL levels (*r* = -0.23) and activity (*r* = -0.27) of the allergic patients but was not as significant as with peripheral blood eosinophilia. High plasma MBL levels might be contributing to the increased recruitment of eosinophils, possibly through the release of chemotactic factors, such as C3a and C5a, during lectin pathway activation.<sup>1</sup>

To provide experimental evidence for the clinical observations, we estimated plasma MBL levels before and after the allergen and PBS challenge in a *fumigatus*-

hypersensitive mice ( $n = 8$ ) and control mice ( $n = 8$ ), respectively, prepared and evaluated as earlier.<sup>7</sup> All procedures involving animal handling and care were approved by the institutional animal use and care committee. The functional activity of both homologous forms of plasma MBL in rodents, mMBL-A and mMBL-C, was quantified before and after 72 hours of last sensitization (28 days immunization schedule) with *A fumigatus* allergens/PBS in the same mice by using biotinylated mAb anti-mouse MBL (13H6 for MBL-A and 14D12 for MBL-C) and streptavidin horseradish peroxidase.<sup>8</sup> There was a significant increase of 56% (4.2%) in the mean mMBL-A level of *A fumigatus*-hypersensitive mice 72 hours after the last sensitization with *A fumigatus* allergens-antigens ( $P < .05$ ), indicating that the allergen challenge might be leading to increased functional levels of MBL in the sensitized mice. In agreement with an earlier report, the mean mMBL-C level, however, showed only a marginal increase of 6% (SD, 0.68%) in the sensitized mice.<sup>9</sup> The mean mMBL-A and mMBL-C levels of the control mice did not show any significant change.

Because polymorphisms in the collagen region of MBL are important determinants of MBL levels and complement activity in the plasma,<sup>3</sup> it is interesting to note that the A allele of one of the novel polymorphisms, G1011A in intron 1 of the *MBL* gene, was found to be significantly associated with high plasma levels of functional MBL. Also, allergic patients homozygous for the A allele had significantly higher peripheral blood eosinophilia and lower percent predicted FEV<sub>1</sub> than the patients homozygous for the other G allele (Kaur et al, unpublished results).

Although increased plasma levels of functional MBL in the patient groups and sensitized mice and their significant correlation with hypereosinophilia suggests a possible role of MBL in the pathophysiology of allergy, in view of MBL being documented as a weak acute-phase protein, such an inference necessitates further investigation.<sup>3</sup> Furthermore, these observations caution the use of MBL replacement therapy in patients with a history suggestive of asthma. Although MBL levels are undetectable in the bronchoalveolar lavage fluid of normal mice, they are increased significantly during pulmonary infection.<sup>10</sup> Hence MBL levels in the bronchoalveolar lavage fluid of allergic patients might be a sensitive marker of allergic airway diseases than their plasma MBL levels.

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## CC chemokine receptors CCR1 and CCR4 are expressed on airway mast cells in allergic asthma

To the Editor:

Mast cells have been implicated as essential inflammatory cells in the asthmatic process. Thus, mast cell numbers are increased in the lungs of both atopic and nonatopic variants of asthma, and the extent of degranulation of the cells is directly related to disease severity.<sup>1</sup> The accumulation of mast cells in the airway is regulated by migration of either progenitor cells circulating in the blood or by relocalization of mature mast cells within the