

Regulation of disease susceptibility: Decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis

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The development of restricted cytokine profiles by subsets of CD4⁺ T cells is a pivotal point in the regulation of immune responses. T cells producing Th1 cytokines (IL-2 and interferon- γ) induce cell-mediated immunity, whereas T cells producing Th2 cytokines (IL-4, IL-5, and IL-10) play a prominent role in the induction of humoral immunity. We examined a group of patients with multiple sclerosis, a disease caused by excess production of Th1 cytokines in myelin-reactive T cells, and control patients with noninflammatory neuroconvulsive disorders, for the presence of allergic disease, which is caused by excess production of Th2 cytokines in allergen-specific T cells. The patients with multiple sclerosis had significantly fewer allergic symptoms, a lower number of positive allergen-specific IgE test results, and lower composite allergy indexes than control subjects. These results demonstrate that the prevalence of IgE-mediated allergic disease is decreased in a group of patients with multiple sclerosis and support the hypothesis that genetic factors that promote susceptibility to Th1-mediated inflammatory disease in human beings protect against the development of Th2-mediated disease. (J Allergy Clin Immunol 1996;97:1402-8.)

Key words: Cytokine regulation, CD4⁺ T cells, autoimmune disease, Th1, Th2, allergic disease, multiple sclerosis

Immune responses are critically regulated by the production of specific cytokines in CD4⁺ helper T cells. The production by CD4⁺ T cells of interferon- γ (IFN- γ) and IL-2 (Th1 cytokines) is associated with the development of cell-mediated immunity and delayed-type hypersensitivity responses.^{1,2} In contrast, the production of a different set of cytokines (Th2 cytokines, i.e., IL-4 and IL-13, essential for the production of IgE^{2,3}), IL-5 (es-

Abbreviations used

IFN- γ : Interferon- γ

MS: Multiple sclerosis

sential for the differentiation of eosinophils⁴), and IL-10 (inhibits IFN- γ production and promotes B-cell and mast cell differentiation³) is associated with the development of humoral immunity. Th1 cytokines (IFN- γ and IL-2) inhibit the production of Th2 cytokines (IL-4, IL-5, and IL-10), whereas Th2 cytokines inhibit the production of Th1 cytokines, thus accentuating the production of polarized cytokine profiles during immune responses.² A balance, however, in the production of these opposing cytokine sets is critical, because overproduction of Th1 cytokines is thought to result in autoimmune inflammatory diseases, such as multiple sclerosis (MS) and diabetes mellitus; whereas overproduction of Th2 cytokines results in allergic

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inflammatory disease, such as asthma and allergic rhinitis, or ineffective immunity to intracellular pathogens.⁴⁻⁹

Both autoimmune and allergic inflammatory diseases are genetically determined, presumably by polymorphisms or mutations at multiple genetic loci¹⁰⁻¹³; but specific genes, gene products, and molecular mechanisms that cause disease in some individuals but not others are not yet defined. Genes regulating cytokine synthesis in CD4⁺ T cells may be involved in this genetic predisposition to autoimmune and allergic inflammatory diseases by enhancing the development of Th1 or Th2 cytokine profiles. Indeed, elevated production of IL-4 and IgE antibody in allergic patients is associated with polymorphisms on chromosome 5q31.1, a region encoding for the Th2 cytokines IL-4, IL-5, IL-9, IL-13, and the p40 subunit of IL-12, a cytokine recently found to be extremely important in inducing IFN- γ synthesis.^{11, 14, 15} In addition, studies in rodents indicate that murine analogs of human inflammatory diseases occur more frequently in rodent strains predisposed to the production of Th1 cytokines.¹⁶ However, it is not known whether in human beings, a predisposition to produce a certain profile of cytokines in a given individual is a global pattern, affecting cytokine production in all immune responses and predisposition to multiple diseases.

If the regulation of cytokine synthesis is a major determinant of autoimmune inflammatory versus allergic inflammatory diseases and if the predisposition to a specific cytokine profile is genetically determined, then one would expect that allergic inflammatory disease (a Th2-mediated problem) and autoimmune inflammatory disease (a Th1-mediated problem) should not both occur in a given individual. Therefore the purpose of our study was to determine whether individuals with a predilection toward the production of a Th1 cytokine-mediated inflammatory disease, MS, have a reduced likelihood of developing a Th2 cytokine-mediated disease, allergy. Here we show that patients with MS do indeed have a decreased prevalence of IgE-mediated allergic disease and demonstrate that two distinct problems, autoimmune inflammatory and allergic inflammatory diseases, tend to be mutually exclusive in this single population of patients. These studies indicate that the immune system in such patients with MS is predisposed to the production of Th1 but not Th2 cytokines in response to at least several different antigens, including autoantigens and allergens.

METHODS

Thirty-seven patients with "clinically definite" MS, determined on the basis of clinical and radiologic criteria,¹⁷ and followed up in the Stanford University Hospital Neurology Clinics, were asked in the spring of 1994 to participate in a study involving a questionnaire and blood sampling. Thirty-five patients (95%) returned questionnaires, and of those, 24 (69%) consented to venous blood sampling and completed the study. The 11 patients who were unable to appear for blood sampling declined because of transportation and health issues. These 11 patients with MS, however, did complete questionnaires, which indicated that they did not differ from the other 24 patients with MS with regard to allergic symptom scores or frequency of a positive family medical history of atopy ($p > 0.1$). Eighteen control patients with noninflammatory neuroconvulsive disorders from the Stanford Neurology Clinics who were having blood drawn to assess anticonvulsant levels were selected during the same period. The patients with MS included 20 Caucasians, three Hispanics, and one Asian. The control subjects included 14 Caucasians and four Hispanics. The study was approved by the Human Subjects Committee, and informed consent was obtained from all subjects.

Total serum IgE levels were determined by ELISA as previously described,¹⁸ and allergen-specific IgE to 36 different allergens was determined by using the MAST CLA allergy system (MAST Immunosystems, Mountain View, Calif.), which is a nonisotopic modification of the RAST method.¹⁹ The allergens included cat dander, dog dander, cockroach, dust mite, *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., Bermuda grass, Johnson grass, Kentucky bluegrass, oak, olive, acacia, ash, birch, elder, cedar, cottonwood, sycamore, cocklebush, and plantain pollens. Results for each allergen were reported in a semi-quantitative fashion as a score between 0 and 4, with 0 indicating negative; 1+, low; 2+, intermediate; 3+, strong; and 4+, a very strongly positive reaction. Allergen-specific IgE assays have been found to correlate directly with allergen challenge results, skin test reactivity, and symptom scores.²⁰⁻²² The total MAST score for each individual was computed from the sum of the scores for each of the 36 allergens. Clinical features of allergic disease were assessed by a questionnaire evaluating allergic symptoms (sneezing, congestion, pruritus, rhinorrhea, ocular symptoms) and allergy medication use on a scale of 0 to 10. Family medical history of allergic disease was also recorded. Because medical history alone does not establish the diagnosis of allergic disease, a composite allergy index was designed to reflect the degree of allergic disease on the basis of measures of IgE hypersensitivity and clinical symptoms.²³ A number of 0 to 10 was used to codify the total MAST scores and allergy symptom scores, and a number from 0 to 5 was given for the total serum IgE, which has the lowest specificity of the measures. The composite allergy index was computed from the sum of these three numbers.

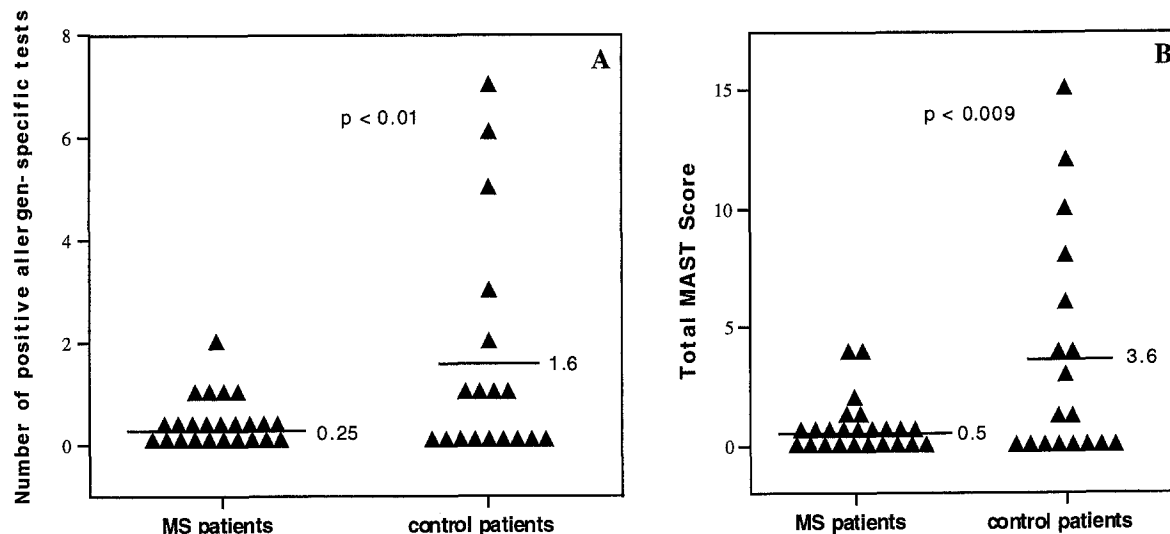


FIG. 1. A, The number of positive allergen-specific IgE test results in a panel of allergens is lower for patients with MS compared with control patients. Each triangle represents the number of positive allergen-specific IgE test results for a single patient. Mean number was 0.25 positive test results for patients with MS and 1.6 positive test results for control patients ($p < 0.01$, Mann-Whitney test). **B,** Total MAST scores for allergen-specific IgE determinations are lower in patients with MS compared with those of control patients. Each triangle represents the score for a single patient. Mean total score was 0.5 for patients with MS and 3.6 for control patients ($p < 0.009$, Mann-Whitney test).

The Mann-Whitney nonparametric U test was used for comparisons between groups, and comparisons of proportions were done by chi square analysis.

RESULTS

Twenty-four patients with MS (group 1 with a mean age of 49 ± 9 years) and 18 control patients (group 2 with a mean age of 41 ± 16 years) completed both the questionnaire and blood sampling. Both groups had similar proportions of male and female subjects (group 1: 9 male and 15 female subjects; group 2: 8 male and 10 female subjects, not significantly different by chi square analysis, $p > 0.46$). Other factors known to affect IgE synthesis did not differ: one patient with MS smoked (which could increase serum IgE levels), four patients in group 1 (20%) were Hispanic or Asian (which could increase the likelihood of allergic disease), and four patients in group 2 (22%) were Hispanic. No patients were receiving immunosuppressive drugs or systemic corticosteroids. All patients were recruited from the Stanford University Hospital Neurology Clinics to minimize differences between the groups in terms of socioeconomic status or place of residence.

Allergen-specific IgE was determined for 36 different allergens including pollens, dust mite, cat, dog, and molds (see Methods). The most common

positive test results in both groups were for dust mite, cat, and grass pollens. As expected, less than half of the patients in both groups had positive test results (Fig. 1, A). However, five of 18 patients in group 2 (control) (28%) had allergen-specific IgE to two or more allergens (a commonly used definition of allergic disease²³), consistent with the fact that the prevalence of allergic disease in the general population is about 15% to 40%.^{22, 24-27} In contrast, only one of the patients in group 1 (4%) had allergen-specific IgE to two or more allergens ($p < 0.03$). The mean number of positive MAST test results for each subject in group 1 (patients with MS) was 0.25, much lower than that for group 2, which was 1.6 ($p < 0.01$), as shown in Fig. 1, A.

Because the likelihood of having allergic disease is greater in individuals with a greater number of allergen-specific test results and in those with more strongly positive results (the IgE determinations for individual antigens are reported on a scale from 0 to 4, with higher scores representing more clinically significant values),¹⁹ the scores for all antigens were summed for each patient to produce a total MAST score (Fig. 1, B). The mean total MAST scores were 0.5 for group 1 and 3.6 for group 2 ($p < 0.009$).

Although the mean total serum IgE level in group 2 (61 U/ml) was greater than that for group

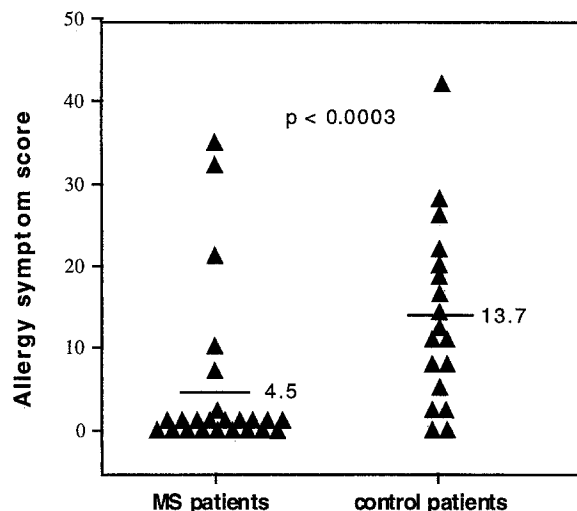


FIG. 2. Allergy symptom scores for patients with MS are lower than those for control patients. Each triangle represents the score for a single patient. The mean allergy symptom score for patients with MS was 4.5, whereas the mean score for the control patients was 13.7 ($p < 0.0003$, Mann-Whitney test).

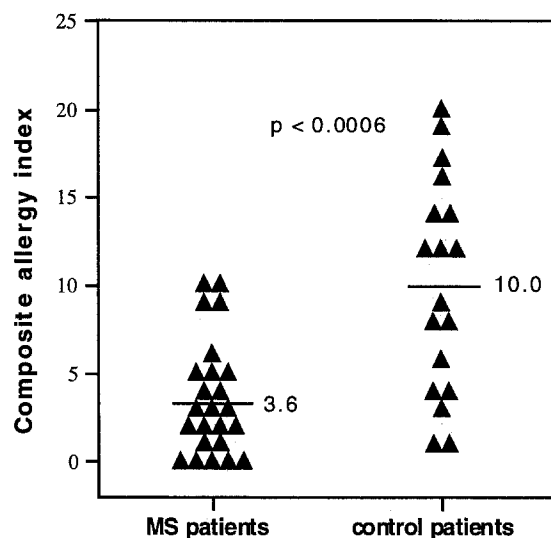


FIG. 3. Composite allergy index is lower in patients with MS than in control patients. Composite allergy index incorporated symptom scores and serologic studies. Each triangle represents the index for a single patient. The mean index in the group of patients with MS was 3.6 compared with 10.0 for the control group ($p < 0.0006$, Mann-Whitney test).

1 (35 U/ml), it was not significantly different ($p > 0.05$) (data not shown). The mean total serum IgE levels for both groups were in the normal range, reflecting the fact that most of the patients did not appear to have allergic disease, as one would expect, and the fact that total serum IgE concentrations have poor sensitivity and are of limited value as a screening test for allergic disease. The family medical history was positive for atopy in 47% of the control patients but was positive for atopy in only 25% of the patients with MS. Although this difference was in the expected direction, it did not reach statistical significance in our small populations, most likely because family medical history of atopy is not a strong indicator of atopy in a given individual (in contrast to immediate skin test reactivity).

Allergic symptom scores were significantly lower for group 1 (mean, 4.5; range, 0 to 32) compared with group 2 (mean, 13.7; range, 0 to 42) ($p < 0.0003$) (Fig. 2), consistent with the results for allergen-specific IgE levels. In a given individual, positive MAST test results correlated with elevated allergic symptom scores. Because the diagnosis of allergic disease in any given patient is based on an evaluation of allergic symptoms and on the presence of allergen-specific IgE, a composite allergy index was computed for each patient on the basis of percentile ranks of MAST scores, symptom scores, and total serum IgE. The mean

composite allergy index for patients in group 1 was 3.6, which was significantly lower than that for patients in group 2, whose mean allergy index was 10.0 ($p < 0.0006$) (Fig. 3).

DISCUSSION

These results demonstrate that the prevalence of allergic disease is greatly reduced in a population of patients with MS compared with a control group, on the basis of clinical evaluation in conjunction with serologic analysis for the presence of allergen-specific IgE. We demonstrated that patients with MS had a significantly lower total number of positive MAST test results for allergen-specific IgE and significantly lower total MAST scores. Furthermore, the patients with MS had significantly lower symptom scores and lower composite allergy indexes, which incorporated both symptom scores and serologic measurements. To our knowledge, this is the first report describing a decreased prevalence of IgE-mediated allergy to inhaled environmental antigens in patients with the autoimmune inflammatory disease, MS.

In this study, the number of patients in each group was small, and therefore the results must be confirmed by studies with larger numbers of patients. However, in spite of the small size of this study, we believe that our results are statistically

and biologically significant because the prevalence of allergic disease, affecting 15% to 40% of the general population,^{22, 24-27} is quite high and can be estimated with statistical accuracy in a relatively small population of patients with MS. In contrast, because the prevalence of MS in the general population is extremely low, generation of statistically significant data in a converse study, showing that patients with allergic disease have a reduced prevalence of MS, would be extremely difficult to achieve and would require examination of several thousand allergic patients in order to observe the few patients who might have MS. In our study, however, we could clearly confirm that allergic disease affects a large fraction of the general population (28% of the control patients had allergic disease, as defined by two or more positive MAST test results), and in addition, we could determine that allergic disease was rare in the patients with MS (4% of the patients with MS had allergic disease as determined by the same criteria). The relatively low prevalence of allergic disease in patients with MS was clearly different from that of the control group and significantly lower than that estimated in previous studies of the general population.^{22, 24-27} Although the mean age of patients in the experimental group was statistically greater than that of the control group ($p = 0.04$) (which would tend to decrease the incidence of positive MAST test results and allergic rhinitis, but not asthma, in the population with MS),^{25, 26} it is very unlikely that age by itself would dramatically decrease the prevalence of allergic disease to well below that seen in the general population.

The prevalence of another autoimmune inflammatory disease, diabetes mellitus, has been previously suggested to be decreased in patients with asthma.^{28, 29} However, this relationship has not been confirmed by many other studies.³⁰⁻³² The discrepancy in results may be due to difficulty in defining diabetes or identifying subjects who were truly atopic, because some studies relied only on questionnaires rather than skin testing or RAST for diagnosis of allergy. In addition, it is possible that the predisposition to production of Th1 cytokines in patients with diabetes is not as pronounced as it is in patients with MS. Predisposition to the production of polarized Th1 or Th2 cytokine profiles is not absolute in that patients with polarized cytokine profiles are not immunodeficient; and cytokine production in a particular individual can be altered or reversed (e.g., by adjuvants, route of exposure to antigen, antigen dose, and cytokine microenvironment).² Clearly, an occasional patient

with MS can develop allergies. Therefore a more definitive demonstration of reduced prevalence of allergic disease in patients with diabetes mellitus may require larger numbers of patients or more specific and clear-cut criteria for the diagnosis of diabetes mellitus and/or allergy.

Our study fully supports the idea that in human beings, regulation of cytokine synthesis is a significant factor in the development of particular diseases, and in addition, indicates that the regulation of cytokine synthesis may affect the predisposition to multiple diseases in an individual. The concept of CD4⁺ T-lymphocyte heterogeneity in disease pathogenesis is well established in rodent systems, and the existence of Th1 and Th2 cells in human beings is widely accepted, though somewhat more controversial.³³ Production of restricted cytokine profiles in human lymphocytes has previously been shown to be a pivotal factor in the development of autoimmune, allergic, and infectious diseases⁴; but there is no previous direct evidence in human beings that an individual may be predisposed to or protected from several distinct diseases states. Thus MS has been shown to be an organ-specific autoimmune disease occurring in genetically susceptible individuals and to be characterized by the presence in the cerebrospinal fluid of myelin-specific T cells producing IFN- γ but not IL-4.^{7, 34, 35} On the other hand, allergic disease is an inherited disorder in which allergen-specific CD4⁺ T cells overproduce IL-4, which then enhances the synthesis of allergen-specific IgE.^{18, 36} Nonallergic individuals, however, possess allergen-specific T cells that produce Th1 rather than Th2 cytokines.^{33, 37-39} Our results validate the Th1/Th2 concept in human beings and support the idea that cytokine production is critically related to disease pathogenesis.

Most importantly, our results indicate that the genetic predisposition to the production of a particular polarized cytokine profile in a given individual is a global pattern, which predisposes that individual to the development of the same polarized cytokine profile in response to a broad range of, if not all, antigens to which he or she is exposed, including autoantigens and allergens. The predisposition to a specific cytokine profile and a specific disease in a given individual affects cytokine production in the development of or protection from other apparently unrelated diseases. Thus the predisposition to Th1 cytokine production in MS also results in the development of allergen-specific T cells producing Th1 cytokines, which inhibit production of allergen-specific IgE and protect against the development of allergy.

The observation that patients with MS might be protected from the development of allergic disease has far-reaching implications, because it changes our understanding of a broad range of human diseases. For example, if genetic regulation of cytokine profiles is important in defining susceptibility to autoimmune, allergic, and infectious diseases, one would predict that patients with MS and other inflammatory diseases might have some degree of protection against the development of progressive infection with *Mycobacteria leprae*, *Leishmania major*, or HIV, because immunity against such microorganisms requires the production of large quantities of Th1 cytokines. In contrast, allergic individuals may have a degree of protection from developing organ-specific autoimmune inflammatory diseases, because their T cells are not likely to overproduce the Th1 cytokines characteristic of disease-producing autoantigen-specific T cells. However, such allergic individuals may be predisposed to the development of progressive infection with *Mycobacteria* spp., *Leishmania* spp., or HIV, because production of Th2 cytokines exacerbates infection with these microorganisms. We are currently investigating these possibilities.

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