

Reduced prevalence of allergic disease in patients with multiple sclerosis is associated with enhanced IL-12 production

Lily Tang, BA, Suwat Benjaponpitak, MD, Rosemarie H. DeKruyff, PhD, and Dale T. Umetsu, MD, PhD *Stanford, Calif*

Background: We previously showed that the prevalence of allergic disease is decreased in patients with multiple sclerosis (MS); however, the mechanisms that explain this finding have not previously been defined.

Objectives: We have demonstrated that protection of patients with MS from allergic disease may be caused by the production in monocytes from these patients of elevated quantities of IL-12 compared with that observed in monocytes from individuals with allergies.

Methods: Purified monocytes from peripheral blood of subjects with or without allergies and from individuals with MS were directly stimulated with *Staphylococcus aureus* Cowan strain I in the absence of T cells. IL-12 was quantitated by a sensitive reverse transcription, competitive PCR.

Results: IL-12 production was 5-fold greater in monocytes from patients with MS ($n = 11$) than that from individuals with allergies ($n = 10$) (for subjects with MS, 1.90 ± 0.18 vs 1.24 ± 0.19 $\log_{10}$ fmol/ μ L for individuals with allergies) ($P = .02$). Although the production of IL-12 in monocytes from patients with MS was slightly higher than that from subjects without allergies, this difference was not statistically significant.

Conclusions: IL-12 production in individuals with MS is much greater than in individuals with allergies. Because IL-12 induces T_H1 cytokine synthesis and reduces the production of T_H2 cytokines, which amplify and prolong allergic inflammation, these studies suggest that enhanced IL-12 production may protect individuals with MS from the development of allergy but may predispose such individuals toward autoimmune inflammation in the central nervous system. (*J Allergy Clin Immunol* 1998;102:428-35.)

Key words: Allergy, autoimmune disease, IL-12, multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system associated with the

Abbreviations used

MS:	Multiple sclerosis
SAC:	<i>Staphylococcus aureus</i> Cowan strain I
rh:	recombinant human

development of autoreactive, myelin-specific T-helper cells producing T_H1 cytokines such as IFN- γ and TNF- β .^{1,2} In contrast, asthma and allergic rhinitis are diseases associated with the development of allergen-specific T-helper cells producing T_H2 cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13, which enhance IgE-mediated hypersensitivity and eosinophilic inflammation in respiratory tracts.³⁻⁵ Individuals with MS appear to be protected from the development of allergic disease; we and others have previously shown that the prevalence of allergic disease is greatly diminished in patients with MS.^{6,7} Both MS and allergic diseases are genetically determined,⁸⁻¹⁰ possibly by genes that affect the regulation of T-helper cell subset development, but specific immunologic mechanisms that might be responsible for lack of allergies in patients with MS are not known.

We examined mechanisms that might explain the reduced prevalence of allergic disease in individuals with MS. We therefore studied the production of IL-12 among individuals with MS and individuals with allergic disease to determine whether differences in IL-12 production might account for the reduced prevalence of allergy in patients with MS. Because the amount of biologically active IL-12 normally produced by monocytes is extremely low, we used a sensitive, competitive PCR to accurately quantitate IL-12 mRNA in monocytes isolated from the peripheral blood of study subjects. In our study, in which monocytes were directly stimulated in the absence of T cells, we demonstrated that monocytes from patients with MS produced much higher quantities of IL-12 than did those from individuals with allergic diseases. Because IL-12 is thought to be the most important T_H1 -inducing factor^{11,12} and because IL-12 can inhibit T_H2 cytokine synthesis, we suggest that enhanced IL-12 production may protect individuals with MS from the development of allergy but predispose such individuals toward autoimmune inflammation in the central nervous system.

From the Division of Immunology and Transplantation Biology, Department of Pediatrics, Stanford University.

Supported by grants RO1-AI26322, RO1-AI24571, and P60-AR20610 from the National Institutes of Health. L.T. was supported by a Howard Hughes Summer Research Fellowship from the Department of Biological Sciences, Stanford University. S.B. was supported by a grant from Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Received for publication April 9, 1998; revised May 19, 1998; accepted for publication May 19, 1998.

Reprint requests: Dale T. Umetsu, MD, PhD, Division of Immunology and Transplantation Biology, Department of Pediatrics, Stanford University, Stanford, CA 94305-5208.

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/91798

TABLE I. Characteristics of study subjects

Characteristics	Subjects with MS	Subjects with allergies	Subjects without allergies
No.	11	10	10
Age (y)†	49.6 ± 4.36*	31.4 ± 2.92*	28.8 ± 1.87*
Male/female	5/6	5/5	6/4
Atopy‡	1 (9.09%)	10 (100%)	0 (0%)
Total IgE (kU/L)†	20.7 ± 12.1*	103.9 ± 23.2*	7.1 ± 1.9*

*Mean ± SEM.

†Atopy was present when individual had history of allergic rhinitis, atopic dermatitis, and/or asthma and had allergen-specific IgE to 2 or more common aeroallergens as measured by solid-phase immunoassay (MAST).

‡P < .05 compared with allergic group.

METHODS

Study population

Eleven patients with MS were selected on the basis of a definitive diagnosis, with criteria established by Poser et al.¹³ These patients were stable or in a state of remission when their blood was drawn. Ten subjects with allergies were selected on the basis of history of allergic rhinitis, asthma, and/or atopic dermatitis, with positive reactions to 2 or more allergen-specific IgE tests in a panel consisting of 36 aeroallergens. Donors without allergies consisted of 10 healthy subjects without any allergen-specific IgE to common aeroallergens. All subjects gave written informed consent, and experimental protocols were approved by the Stanford University Administrative Panel on Human Subjects in Medical Research.

Antigens and cytokines

Staphylococcus aureus Cowan strain I (SAC; Calbiochem, La Jolla, Calif) was used in culture at 15 µg/mL. Recombinant human (rh)IFN-γ (Amgen, Thousand Oaks, Calif) and rhIL-4 (R&D Systems, Minneapolis, Minn) were used at a concentration of 100 U/mL (rhIFN-γ) and 2 ng/mL (rhIL-4).

Preparation of monocytes

PBMCs were isolated from heparinized venous blood of subjects by centrifugation (30 minutes, 2000 rpm) through Ficoll-Hypaque (Sigma, St Louis, Mo). To obtain a purified monocyte population, isolated PBMCs, 5 to 8 × 10⁶ cells/mL, were incubated in 60-mm plastic dishes in Dulbecco's modified Eagle's media (Life Technologies, Inc, Gaithersburg, Md) supplemented with 10% AB+ human serum (Hazleton, Lenexa, Kan), 1 mmol/L sodium pyruvate, 10 µg/mL streptomycin, 100 U/mL penicillin (Applied Scientific, San Francisco, Calif), and 2 mmol/L L-glutamine for 1 hour at 37°C. Nonadherent cells were removed by gently washing the dishes 3 times with warm RPMI 1640 media (Life Technologies, Inc) containing 5% FCS. The remaining adherent cells were then harvested by incubating for 10 minutes with ice-cold PBS and rinsing repeatedly. These cells were composed principally of monocytes and were more than 99% Mac-1⁺, as previously described.¹⁴

Stimulation of monocytes

Monocytes were stimulated for 6 hours in 24-well plastic dishes at 10⁶ cells/mL (Nunc, Naperville, Ill) in complete media in 90% air, 10% CO₂ humidified environment at 37°C with the following conditions: SAC alone, SAC plus rhIFN-γ, and SAC plus rhIL-4. mRNA was purified from activated cells and quantitated by reverse transcription, competitive PCR.

Competitive IL-12 p40 PCR assay

To assess IL-12 production, we analyzed IL-12 mRNA by using a quantitative reverse transcription PCR procedure. The procedure is made much more quantitative and accurate for measuring small changes in mRNA levels by using nonhomologous DNA fragments as competitive internal standards. The competitor IL-12 p40 PCR MIMIC used in this PCR assay was generated with the PCR MIMIC Construction Kit (Clontech, Palo Alto, Calif) according to the manufacturer's instructions. Serial dilutions of known quantities of competitor IL-12 p40 PCR MIMICs (10,000, 500, 100, 50, 10, 1, 0.1, or 0.01 fmol/µL) were added to the PCR reaction tubes containing constant amounts of sample target IL-12 cDNA. The target IL-12 cDNA was generated by reverse transcription of purified mRNA from the monocytes of subjects with MS and subjects with and those without allergies. The tubes contained a total of 50 µL of 20 nmol/L Tris-HCl, pH 8.4, 2 mmol/L MgCl₂, 0.2 mmol/L of each dNTP, 2 U of Taq polymerase (Gibco BRL, Gaithersburg, Md), and 400 nmol/L of primers. The PCR reaction was carried out for 35 cycles (1 minute of denaturation at 94°C, 1 minute of annealing at 63°C, 1 minute of extension at 72°C, and followed by a final extension at 72°C for 10 minutes). Because the PCR MIMIC fragments were designed to contain the same primer templates as the target IL-12 cDNA, both MIMIC and target were coamplified by the same primers in each reaction tube and thus competed during the amplification reaction for the same primers.

Primers used for competitive PCR were as follows: p40 primer 5'-5'-CCAAGAACTTGCAGCTGAAG-3' and p40 3' primer 5'-TGGGTCTATTCCGTTGTGTC-3'. The IL-12 and competitor PCR products were easily resolved by gel electrophoresis (1.8% agarose gels, Gibco BRL, containing 0.2 µg/mL ethidium bromide), because the competitor product is approximately 200 base pairs longer than the IL-12 target cDNA product. The bands in the gels were visualized and photographed with a Gel Doc 1000 (Bio-Rad, Laboratories, Hercules, Calif) under ultraviolet light. Because the amount of competitor template added to each reaction was known, the amount of target IL-12 cDNA template could be assessed by determining which tube produced equal amounts of competitor and target cDNA product. In this reaction tube, the competitor and cDNA competed equally for the primers and therefore equal amounts of product were generated, which was visualized on gel electrophoresis as bands of equivalent intensity.¹⁵ Therefore in this reaction tube the starting concentration of IL-12 cDNA template before PCR was equal to the known concentration of the competing MIMIC DNA template. It should be noted that competitive PCR, with an internal coamplified competitor rather than a separate control such as actin, which would require a different set of primers in

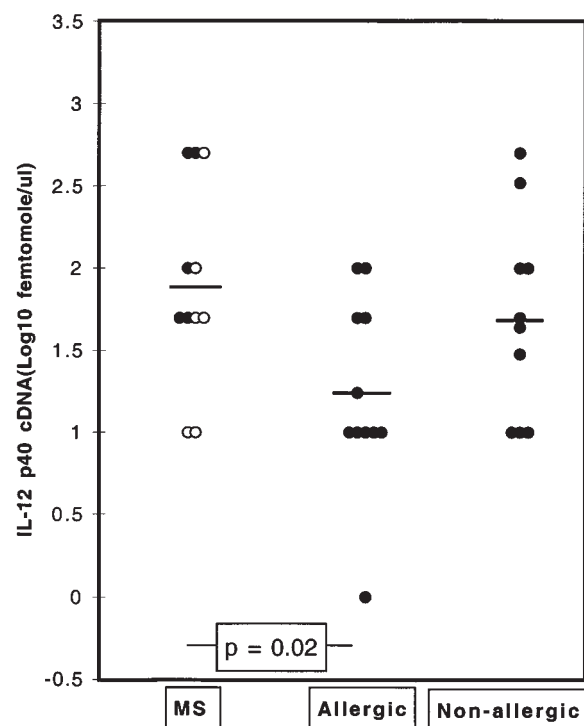


FIG 1. IL-12 production induced with SAC is significantly greater in monocytes from individuals with MS than in those from subjects with allergies ($P = .02$). Monocytes from individuals with MS ($n = 11$), subjects with allergies ($n = 10$), and subjects without allergies ($n = 10$) were stimulated with SAC for 6 hours. IL-12 cDNA was synthesized from extracted total RNA by reverse transcription and quantitated by competitive PCR. Circles represent amount of IL-12 cDNA in $\log_{10}$ fmol/ μ L for single subjects. Among patients with MS, those receiving medical therapy are indicated by open circles ($n = 6$) and patients with MS without medical therapy with filled circles ($n = 5$). Mean values are represented by horizontal bars.

a separate tube with different efficiencies of amplification, gives improved accuracy and reproducibility and decreased variability.

RNA isolation, cDNA synthesis, and competitive PCR for quantitation of IL-12 cDNA

For analysis of IL-12 p40 mRNA expression, total RNAs were extracted from the stimulated cells with the RNeasy Total RNA Kit (Qiagen, Hilden, Germany). The first strand of IL-12 cDNA was synthesized from total RNA (1 μ g) with 2.5 U/ μ L Moloney murine leukemia virus–derived reverse transcriptase (Perkin-Elmer, Norwalk, Conn), 1 mmol/L each of the dNTPs (dATP, dCTP, dGTP, and dTTP; Gibco BRL), RNase inhibitor (1 U/ μ L; Perkin-Elmer), Oligo d(T)16 (2.5 μ mol/L; Perkin-Elmer), 10 $\times$ PCR buffer (500 mmol/L KCl, 200 nmol/L Tris-HCl, pH 8.4; Gibco BRL), and $MgCl_2$ (5 nmol/L) to a total volume of 20 μ L. The reverse transcription PCR was performed with the PCR DNA engine (Peatier Thermal Cycler model PTC-200, MJ Research, Watertown, Mass). The RT mix was incubated at 42°C for 15 minutes followed by 99°C for 5 minutes to inactivate the reverse transcriptase.

Quantitation of serum IgE

The amount of total serum IgE was determined by ELISA. The ELISA was performed in 96-well microtiter plates (Dynatech Lab-

oratories Inc, Chantilly, Va) coated with 2 anti-human IgE mAbs (1 μ g/mL; obtained IRBM, Dr Ruben Siraganian, National Institute of Dental Health, Bethesda, Md). After washing with PBS with 0.1% Tween 20, the plates were blocked with a 0.1% BSA solution for 1 hour and washed, and 0.1 mL of appropriately diluted serum was added to the wells. After 18 hours, the plates were again washed, and biotinylated goat anti-human IgE (Tago Inc, Burlingame, Calif) was added for 120 minutes. The plates were washed and horseradish peroxidase–labeled streptavidin (Southern Biotech Associates, Birmingham, Ala) was added for 60 minutes. Bound enzyme was quantitated with the substrate *o*-phenylene diamine (Sigma Chemical Co) for 10 minutes, and the absorbance at 493 nm was read on a SpectraMax (Molecular Devices Corp, Sunnyvale, Calif) automatic ELISA reader. Standard curves for IgE were performed with purified IgE (Pharmacia AB, Uppsala, Sweden). The lower limit of this assay is 0.06 IU/mL.

Quantitation of serum allergen-specific IgE

Patient serum allergen-specific IgE to 36 different allergens was determined with the MAST CLA allergy system (MAST Immunosystems, Mountain View, Calif), which is a nonisotopic modification of the RAST method. The MAST CLA allergen-specific IgE assay was performed by filling a MASTpette test chamber with patient serum. IgE in the serum bound to the allergen-coated cellulose threads during incubation. The test chamber was then washed with buffer to remove unbound serum components. Enzyme-labeled anti-IgE was then added to the chamber and coupled with the serum IgE bound to the threads. After a second wash, the test chamber was filled with a photoreagent mixture that reacted with the labeled antibody to produce chemiluminescence. The luminometer measured light emission in luminescence units. The amount of light emitted by each thread was directly proportional to the amount of allergen-specific IgE in the patient serum. Results for each allergen were reported in a semiquantitative 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. The score of 2+ or more was considered positive response of allergen-specific IgE. 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.

Statistical analysis

The data are presented as mean $\pm$ SEM of the combined experiments. Student's two-tailed *t* test for independent means was used to determine significant differences ($P < .05$). Because of the great dynamic range of the assay (6 $\log_{10}$ dilutions), the "raw data" values for IL-12 had a very great spread. Therefore we "normalized" the raw data by taking the log transformation of the data. As a result, the statistical calculations were performed on the log-transformed values.

RESULTS

Patient characteristics

The study population consisted of 31 subjects, including 11 individuals with MS, 10 with allergic disease, and 10 subjects without allergies. The characteristics of the subjects are described in Table I. In this study, a subject was defined as atopic if he or she had a history of allergic diseases (allergic rhinitis, atopic dermatitis, and/or allergic asthma) and had allergen-specific IgE to 2 or more allergens. Atopy was present in only 1 of the 11 subjects with MS, a prevalence that was similar to that seen in patients with MS in our previous study⁶ and that

Patients with MS produce more IL-12 mRNA than do patients with allergies

Because some of the patients with MS were receiving therapies that could downregulate IL-12 production (eg, corticosteroids or IFN- β), we separated those patients from those not receiving therapy. IL-12 production did not differ in patients with MS who were or were not receiving therapy. In addition, IL-12 production in patients with progressive MS did not differ from that in patients with stable MS (progressive MS: 2.13 ± 0.98 vs 1.83 ± 0.5 log₁₀ fmol/ μ L in stable MS). Additionally, although IL-12 production in monocytes from individuals without allergies was slightly greater than that from individuals with allergies, the difference was not significant.

IL-12 production in the presence of IFN- γ

IL-12 p40 cDNA(Log10 femtomole/ul)

MS Allergic Non-allergic

$p = 0.01$

Group	IL-12 p40 cDNA (Log10 femtomole/ul)
MS	1.7, 2.0, 2.0, 2.5, 2.5, 2.7, 3.0, 3.0, 3.0, 3.0
Allergic	1.0, 1.0, 1.7, 1.9, 1.9, 2.0, 2.0, 2.0, 2.5, 2.7
Non-allergic	1.7, 1.7, 2.0, 2.0, 2.7, 2.7, 3.0, 3.0

subjects without allergies. Therefore in the presence of IFN- γ , IL-12 production remained significantly greater in monocytes from patients with MS than in monocytes from individuals with allergies: for the MS group, 2.51 ± 0.17 vs $1.88 \pm 0.17 \log_{10}$ fmol/ μ L in the allergic group ($P = .01$). These findings suggested that the reduced production in patients with allergies was not caused by a lack of responsiveness to IFN- γ . Moreover, in the presence of IFN- γ , there was no significant difference in IL-12 production in monocytes from patients with progressive MS versus stable MS (2.47 ± 0.68 vs $2.55 \pm 0.53 \log_{10}$ fmol/ μ L, respectively). In addition, in the presence of IFN- γ , there was no difference in IL-12 production between patients with MS receiving corticosteroids or IFN- β therapy and patients not receiving therapy (2.31 ± 0.6 vs $2.74 \pm 0.43 \log_{10}$ fmol/ μ L, respectively).

IL-12 production in the presence of IL-4

Another possible explanation for the difference in IL-12 production between patients with allergies and patients with MS is that regulation of IL-12 by IL-4 was different in monocytes from these individuals. We therefore examined IL-12 production from SAC-stimulated monocytes in

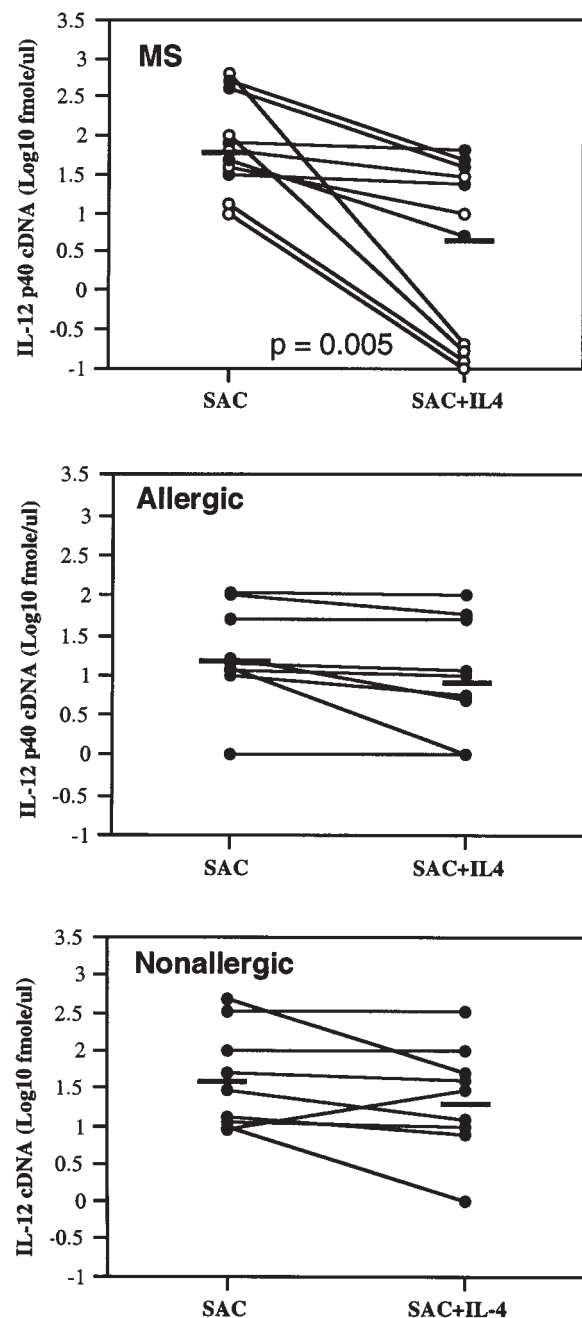


FIG 3. rhIL-4 reduced IL-12 production from SAC-stimulated monocytes of individuals with MS ($P = .005$). Stimulation of monocytes and analysis of IL-12 production were performed as described in Fig 1. Each line represents results with cells from individual donor; mean of data in each group is indicated by horizontal bar.

the presence of added IL-4. Fig 3 shows that exogenous rhIL-4 (2 ng/mL) significantly reduced IL-12 production in monocytes from patients with MS stimulated with SAC, but it did not affect IL-12 production in monocytes from individuals with and those without allergies. Although virtually all patients with MS had a reduction in IL-12 pro-

duction in the presence of exogenous IL-4, patients with MS being treated with corticosteroids or with IFN- β responded most dramatically to exogenous IL-4 and had the most significant reductions in IL-12 production (Fig 3). The difference in IL-12 production in treated and untreated patients with MS in the presence of IL-4 was statistically significant ($P = .01$): IL-12 for MS while receiving therapy, -0.25 ± 1.17 versus 1.45 ± 0.43 log₁₀ fmol/ μ L in untreated patients with MS. These studies suggest that in vivo IL-4 production may inhibit the capacity of MS but not allergic monocytes to produce IL-12 and that in vivo therapies with IL-4 may be beneficial for patients with MS. Whether the enhanced responsiveness to IL-4 of monocytes from patients with MS is caused by reduced prior in vivo exposure to IL-4 is not clear.

DISCUSSION

The results of this study explain the low prevalence of allergic disease in patients with MS.^{6,7} In this report we demonstrated that individuals with MS may be protected from the development of allergic disease because of the elevated production of IL-12 in monocytes of patients with MS compared with that in individuals with allergies. These studies, performed by stimulating purified monocytes from patients with MS and subjects with allergies in the absence of T cells, indicated that individuals differ in the intrinsic capacities of their monocytes to produce IL-12, which may influence the development of diseases such as MS and allergy.

Previous studies have shown that IL-12 production in patients with the progressive form of MS is elevated. However, such studies indicated that elevated IL-12 production was primarily caused by an abnormality in T cells and that monocytes from these patients were only secondarily involved. Thus T cells from patients with progressive but not relapsing and remitting MS, activated with anti-CD3 mAb, could drive non-T cells (including monocytes) from patients with MS and from normal subjects to produce increased IL-12.^{17,18} In addition, investigators found in patients with MS increased CD40 ligand expression by activated CD4⁺ T cells, which enhanced T-cell-dependent IL-12 production.¹⁷ Because IFN- γ (in this case produced by T cells) greatly enhances IL-12 production, these results suggested that elevated IL-12 production in patients with MS was secondary to T cells already committed to produce T_H1 cytokines, particularly in patients with the progressive form of MS.

In contrast, our studies examined monocytes directly rather than in response to activated T cells and determined that monocytes from patients with MS and those with allergy differ in their intrinsic capacities to produce IL-12. By stimulating the monocytes directly with SAC in the absence of T cells and in the absence of CD40/CD40L interactions, we showed that monocytes from patients with MS (most with stable MS) produced greater quantities of IL-12 than did monocytes from individuals with allergies.¹⁹ Although one could argue that the monocytes from patients with MS may have been primed by in vivo expo-

sure to IFN- γ to produce more IL-12, most of our patients with MS had the stable rather than the progressive form of MS, and several were receiving medications that limit IFN- γ and IL-12 production.^{20, 21} Despite this, the quantity of IL-12 produced by MS monocytes was 5-fold greater than that of monocytes from individuals with allergies.

Other investigators have suggested that peripheral blood cells from individuals with allergies produce reduced amounts of IL-12 compared with that of individuals without allergies. In such studies, investigators stimulated whole blood with SAC, without purification or assessment of the numbers of T cells, monocytes, or neutrophils placed in culture.²² It is likely that T cells in such cultures produced cytokines such as of IFN- γ or IL-4, which affected the production of IL-12. In such whole blood cultures, it is not clear whether monocytes were intrinsically deficient in the production of IL-12 or whether the primary difference was cytokine production at the T-cell level, which secondarily influenced IL-12 production. In contrast, our cultures contained purified monocytes and therefore we can conclude that monocytes from patients with MS intrinsically produce greater quantities of IL-12 compared with those from subjects with allergies. Although we showed that monocytes from individuals without allergies had increased IL-12 production compared with those from allergic subjects, this difference was not statistically significant, perhaps because of the small numbers of patients in our study.

Because 20% to 40% of the "normal" general population is atopic, previous studies demonstrating that IL-12 production is elevated in subjects with MS compared with control subjects may be biased by the presence of large numbers of individuals with allergies or even individuals with latent allergies in the control population. We designed our study to specifically exclude such individuals with allergies from our control population because monocytes from these individuals, as shown in Fig 1, produce significantly less IL-12 than those of patients with MS. The exclusion of individuals with allergies from the control population is not easy because mild allergic disease is common and often difficult to detect and diagnose. To avoid such an oversight, all of our subjects were evaluated by questionnaire and by examination for the presence of allergen-specific IgE (MAST assays). The exclusion of individuals with allergies from our control population may explain why IL-12 production in monocytes from our control patients was only slightly lower but not statistically different from that of the patients with MS, whereas other studies showed that IL-12 was increased in the cerebrospinal fluid of patients with MS compared with a control population.^{1, 2} More importantly, in this study, we found that IL-12 production was increased in the monocytes from patients with MS compared with that in monocytes from individuals with allergies. This may be the precise distinction regarding IL-12 production in populations of patients with MS and groups of "normal" control individuals (of which 20% to 40% may have allergies).

Our study is consistent with the idea that excess production of IL-12 can exacerbate MS^{23, 24} and may reduce

the prevalence of allergic disease in this population.^{14, 25} Previous studies have shown that administration of IL-12 in animal models of experimental allergic encephalomyelitis aggravates disease. In addition, expression of IL-12 is seen in acute lesions of MS²⁶ and is associated with the progressive form of the disease, though in this study it was difficult to know whether an underlying increase in IL-12 production caused MS or alternatively resulted from the prominent T_{H1} cytokine synthesis. Nevertheless, IL-12 appears to play a major role in pathogenesis of MS, particularly in disease progression. On the other hand, individuals with allergies may be prone to their disease because of a decrease in IL-12 production. Thus lack of IL-12 may result in increased T_{H2} cytokine production and reduced T_{H1} cytokine production in individuals with allergies. It is possible that reduced IL-12 production "protects" individuals with allergies from the development of MS, though the definitive study, examining thousands of individuals with allergies to identify the few who may have MS, has not been performed.

Intrinsic differences in the capacity of antigen-presenting cells to produce IL-12 as an innate mechanism that regulates T_{H1}/T_{H2} cytokine production and disease susceptibility has been shown previously in mice.²⁷ Macrophages from DBA/2 mice produce much greater quantities of IL-12 than do those of BALB/c mice, resulting in much greater IFN- γ and reduced IL-4 production in T cells from DBA/2 compared with BALB/c mice. This explains the relative resistance of DBA/2 mice and the susceptibility of BALB/c mice to infection with *Leishmania*. As demonstrated by our studies, this mechanism appears to play a role in human disease, although the specific genetic differences that underlie differences in IL-12 production are not yet clear.

We cannot conclude from our study whether elevated IL-12 production in MS compared with that in individuals with allergies begins well before the onset of neurologic symptoms because we measured IL-12 production in patients with MS years after the onset of their disease. It is possible that increased IL-12 production is the result of their disease and that allergic symptoms are abolished in patients with MS after the onset of the disease. We suggest, however, that individuals with MS have an intrinsic increase in their capacity to produce IL-12 above that of individuals with allergies unrelated to disease onset because we previously found that the family medical history in patients with MS was low for atopic disease compared with that of control subjects.⁶ Thus elevated IL-12 production may be an inherited characteristic in families in which some members develop MS.

Both MS and allergic diseases have significant genetic and environmental components that predispose certain individuals toward these diseases.^{8, 9} However, the specific genes, gene products, and molecular mechanisms that cause these diseases are not yet fully defined, although the HLA class II region may play a limited role in predisposition to both diseases.²⁸ An additional candidate gene, in a region that has been shown to regulate susceptibility to allergy and asthma at 5q31.1,^{9, 29, 30} contains the gene for

the p40 subunit of IL-12.³¹ Polymorphisms in this region could explain our results and the reduced prevalence of allergy in patients with MS. Alternatively, environmental factors, for example infection with herpesvirus, may polarize the immune system in such a way as to lead to the development of MS to the exclusion of allergic disease.^{32, 33} Further study of the precise disease mechanisms must be performed to sort out these possibilities.

Finally, although IL-12 production was increased in monocytes from patients with MS while decreased in individuals with allergies, we observed that the elevated production of IL-12 in patients with MS could be significantly reduced by the addition of IL-4 to these cultures. This reduction by IL-4 was preferentially observed in the monocytes from patients with MS and not in those from normal individuals. The effect was greatly enhanced in those patients with MS who were being treated with IFN- β or corticosteroids (Fig 3). This finding supports the use of IL-4 as a therapy for patients with MS and to reduce T_{H1} cytokine production and is consistent with a recent study demonstrating that the local expression of IL-4 by retrovirus-transduced T lymphocytes can delay the onset and reduce the severity of experimental allergic encephalomyelitis in a mouse model.³⁴

In summary, we have shown that the reduced prevalence of allergic disease in patients with MS is associated with elevated production of IL-12 compared with that in individuals with allergies. This has significant implications with regard to the use of IL-12 for the treatment of individuals with allergies because therapy with IL-12 may be effective in reducing the development of allergic response in such patients.

We thank the Multiple Sclerosis Society, Santa Clara County, for help in recruiting patients; Lawrence Steinman for helpful discussion; and MAST Immunosegments, Mountain View, Calif, for help with allergen-specific IgE assays.

REFERENCES

- Navikas V, Link H. Review: cytokines and the pathogenesis of multiple sclerosis. *J Neurosci Res* 1996;45:322-33.
- Drulovic J, Mostarica-Stojkovic M, Levic Z, Stojavljevic N, Pravica V, Mesaros S. Interleukin-12 and tumor necrosis factor- α levels in cerebrospinal fluid of multiple sclerosis patients. *J Neurol Sci* 1997;147:145-50.
- Robinson D, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley A, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298.
- Marshall J, Wen Y, Abrams J, Umetsu D. In vitro synthesis of IL-4 by human CD4⁺ T cells requires repeated antigenic stimulation. *Cell Immunol* 1993;152:18-34.
- Secrist H, DeKruyff R, Umetsu D. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med* 1995;181:1081-9.
- Oro A, Guarino T, Driver R, Steinman L, Umetsu D. Regulation of disease susceptibility: decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. *J Allergy Clin Immunol* 1996;97:1402-8.
- Neukirch F, Lyon-Caen O, Clanet M, Bousquet J, Feingold J, Druet P. Asthma, nasal allergies, and multiple sclerosis. *J Allergy Clin Immunol* 1997;99:270-1.
- Karpuz M, Steinman L, Oksenberg J. Multiple sclerosis: a polygenic disease involving epistatic interactions, germline rearrangements and environmental effects. *Neurogenetics* 1997;1:21-8.
- Postma D, Bleecker E, Amelung P, Holroyd K, Xu J, Panhuysen C, et al. Genetic susceptibility to asthma-bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
- Hsieh C, Macatonia S, O'Garra A, Murphy K. T cell genetic background determines default T helper phenotype development in vitro. *J Exp Med* 1995;181:713-21.
- Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995;13:251-76.
- Manetti R, Parronchi P, Giudizi M, Piccinni M, Maggi E, Trinchieri G, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993;177:1199-204.
- Poser C, Paty D, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983;13:227-31.
- Marshall J, Secrist H, DeKruyff R, Wolf S, Umetsu D. IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human CD4⁺ T lymphocytes. *J Immunol* 1995;155:111-7.
- Gilliland G, Perrin S, Blanchard K, Bunn H. Analysis of cytokine mRNA and DNA: detection and quantitation by competitive polymerase chain reaction. *Proc Natl Acad Sci U S A* 1990;87:2725-9.
- Weiner L, Ellison G. A working protocol to be used as a guideline for trial in multiple sclerosis. *Arch Neurol* 1983;40:704-10.
- Balashov K, Smith D, Khoury S, Hafler D, Weiner H. Increased interleukin 12 production in progressive multiple sclerosis: induction by activated CD4⁺ T cells via CD40 ligand. *Proc Natl Acad Sci U S A* 1997;94:599-603.
- Nicoletti F, Patti F, Cocuzza C, Zaccone P, Nicoletti A, Marco RD, et al. Elevated serum levels of interleukin-12 in chronic progressive multiple sclerosis. *J Neuroimmunol* 1996;70:87-90.
- DeKruyff RH, Umetsu DT. Antigen-driven but not LPS-driven IL-12 production requires direct interaction between CD40 and CD40L. *J Immunol* 1997;158:359-66.
- McRae BL, Picker LJ, van Seventer GA. Human recombinant interferon-beta influences T helper subset differentiation by regulating cytokine secretion pattern and expression of homing receptors. *Eur J Immunol* 1997;27:2650-6.
- Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit interleukin-12 production in macrophages and enhance their capacity to induce IL-4 synthesis in CD4⁺ lymphocytes. *J Immunol* 1997;158:5589-95.
- van der Pouw Kraan T, Boeijs L, Groot E, Stapel S, Snijders A, Kapsenberg M, et al. Reduced production of IL-12 and IL-12-dependent IFN- γ release in patients with allergic asthma. *J Immunol* 1997;158:5560-5.
- Santambrogio L, Crisi G, Leu J, Hochwald G, Ryan T, Thorbecke G. Tolerogenic forms of auto-antigens and cytokines in the induction of resistance to experimental allergic encephalomyelitis. *J Neuroimmunol* 1995;58:211-22.
- Leonard J, Waldburger K, Schaub R, Smith T, Hewson A, Cuzner M, et al. Regulation of the inflammatory response in animal models of multiple sclerosis by interleukin-12. *Crit Rev Immunol* 1997;17:545-53.
- Kim TS, DeKruyff RH, Rupper R, Maecker HT, Levy S, Umetsu DT. An OVA-IL-12 fusion protein is more effective than OVA plus rIL-12 in inducing a Th1-dominated immune response and inhibiting antigen-specific IgE production. *J Immunol* 1997;158:4137-44.
- Windhagen A, Newcombe J, Dangond F, Strand C, Woodroffe M, Cuzner M, et al. Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J Exp Med* 1995;182:1985-96.
- Gieni RS, Fang Y, Trinchieri G, Umetsu DT, DeKruyff RH. Differential production of IL-12 in distinct mouse strains controls Th1 versus Th2 cytokine profiles in primed CD4 lymphocytes. *Intern Immunol* 1996;8:1511-20.
- Compston D. Genetic factors in the aetiology of multiple sclerosis. In: McDonald W, Silberberg D, editors. *Multiple sclerosis: BMR Neurology*. London: Butterworths; 1986. p 56-73.
- Marsh D, Neely J, Breazeale D, Ghosh B, Freidhoff L, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
- Meyers D, Postma D, Panhuysen C, Xu J, Amelung P, Levitt R, et al. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994;23:464-70.
- Sieburth D, Jabs E, Warrington J, Li X, Lasota J, LaForgia S, et al. Assign-

- ment of genes encoding a unique cytokine (IL12) composed of two unrelated subunits to chromosomes 3 and 5. Genomics 1992;14:59-62.
32. Lindegard B. Disease associated with multiple sclerosis and epilepsy: a population cohort study of 159,200 middle-aged, urban, native Swedes observed over 10 years(1970-79). Acta Neurol Scand 1985;71:267-77.
33. Saltine S, Bert R, Salem N, Psychorrhea P, Flamboyant L, Celebres P, et al. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV6 DNA. Nat Med 1997;3:1394-7.
34. Shaw M, Lorens J, Dhawan A, DalCanto R, Tse H, Tran A, et al. Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. J Exp Med 1997;185:1711-4.

O *N THE MOVE?*

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

*Please send your change of address notification at least six weeks before your move to ensure continued service.
We regret we cannot guarantee replacement of issues missed due to late notification.*

JOURNAL TITLE:

Fill in the title of the journal here. _____

OLD ADDRESS:

Affix the address label from a recent issue of the journal here.

NEW ADDRESS:

Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:

Periodical Subscription Services
Mosby, Inc.
11830 Westline Industrial Dr.
St. Louis, MO 63146-3318

OR FAX TO:

314-432-1158



OR PHONE:

1-800-453-4351
Outside the U.S., call
314-453-4351