

Production of interleukin 10 and transforming growth factor β in concomitant allergy and autoimmunity

Sanders K. Chai, MD, MPH*; Gaylene M. Altman, RN, PhD†; Maria Yazdanbakhsh, PhD‡; Joyce Tsuji, MS†; Laura Godat, BS§; and Tim K. Takaro, MD, MPH, MS*

Background: The immunologic relationship between T_H1 -type autoimmune disorders and T_H2 -type allergic disorders and the role of T-cell regulation in humans is as yet unclear. The regulatory cytokine production capacity of individuals with concomitant allergy and T_H1 -type autoimmunity may provide insight into the role of T-cell regulation in both disorders.

Objectives: To examine the production capacity of interleukin 10 (IL-10) and transforming growth factor β (TGF- β), 2 regulatory cytokines, in individuals with concomitant allergic rhinitis and T_H1 -type autoimmune diagnoses and to compare that capacity with that in individuals with allergic rhinitis only and individuals with neither diagnosis.

Methods: Seventeen case subjects and 17 age-, sex-, and ethnicity-matched controls with allergic rhinitis only were recruited from an allergy clinic. Fourteen matched controls with neither diagnosis were recruited from the general population. Peripheral blood mononuclear cells were obtained and cultured with and without mitogen stimulation (lipopolysaccharide and phytohemagglutinin). Cytokine levels from culture supernatants were measured by enzyme-linked immunosorbent assay.

Results: Cases with allergic rhinitis and autoimmune diseases had significantly lower unstimulated day 3 IL-10 levels compared with controls with allergic rhinitis only ($P = .05$) and significantly lower stimulated day 5 TGF- β levels compared with controls with neither diagnosis ($P = .02$). Cases had consistently lower regulatory capacity compared with both control groups, as measured by an additive index using IL-10 and TGF- β levels.

Conclusion: Individuals with concomitant allergic rhinitis and T_H1 -type autoimmune disorders have a lower regulatory cytokine production capacity than individuals with allergic rhinitis only and those with neither diagnosis.

Ann Allergy Asthma Immunol. 2005;94:279–285.

INTRODUCTION

The causes of the changing prevalence of autoimmunity and allergies in the developed world are unknown. The T_H1/T_H2 paradigm, which refers to the differentiation pattern of T-helper lymphocyte development, has been used to describe the immunologic response patterns seen in both categories of illness.¹ Allergies and allergic asthma generally exhibit a T_H2 -type deviation. Conversely, autoimmunity, depending on the exact illness, can induce either a T_H1 response, reflecting a cell-mediated pathophysiology (as in rheumatoid arthritis, multiple sclerosis, type 1 diabetes mellitus, and Crohn dis-

ease), or a T_H2 -type response, reflecting an antibody-mediated pathophysiology (as in systemic lupus and ulcerative colitis).

The simple dichotomous T_H1/T_H2 model is being challenged by recent work in T-cell regulation and tolerance, autoimmunity and allergy epidemiology, and tuberculosis immunology. A population of thymically derived T-helper cells that express high CD25 levels has been found to have potent immunosuppressive capacity.² The activation of these CD4⁺CD25^{high} T cells has been observed to be interleukin 10 (IL-10) and transforming growth factor β (TGF- β) dependent, and these T cells often act synergistically with these cytokines in suppressing T-effector cell activity and proliferation.² It is hypothesized that both these regulatory cytokines are secreted by peripherally differentiated T-regulatory cells (Treg) (in contrast to thymically derived CD4⁺CD25^{high} cells), which, in humans, have yet to be identified. In total, these markers of T-cell regulation and tolerance have been associated with the suppression of autoimmunity and allergic disease in humans.^{3–6}

Supporting a direct association between allergies and autoimmunity are recent epidemiologic studies^{7,8} that show that an allergy or asthma diagnosis actually increases the risk of being diagnosed as having some T_H1 -type autoimmune disorders and vice versa. In these studies, type 1 diabetes mellitus, rheumatoid arthritis, Crohn's disease, and thyroiditis

* Department of Occupational and Environmental Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, Washington.

† Biobehavioral Nursing and Health Systems, School of Nursing, University of Washington, Seattle, Washington.

‡ Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands.

§ Asthma Inc, Seattle, Washington.

This study was supported in part by grant P30ES07033 from the University of Washington's National Institute of Environmental Health Sciences-sponsored Center for Ecogenetics and Environmental Health, training grant T32ES07262 from the National Institute of Environmental Health Sciences, and an unrestricted educational grant from Medpointe Pharma.

Received for publication July 13, 2004.

Accepted for publication in revised form September 27, 2004.

were the T_H1-type diseases that had significant associations with atopy.

Autoimmunity and allergies at a population level are associated with a reduced incidence of infectious diseases. The hygiene hypothesis is used to explain this phenomenon.¹ In this hypothesis, the increasing incidence of autoimmunity and allergies is a result of decreased microbial stimulation, stimulation that would have occurred had there been appropriate infections in an individual's early life. Thus, tuberculosis, a purported T_H1-type provateur, has been reported as having a decreasing incidence that correlates with the rise in allergies and autoimmunity.⁹ More recent studies,^{10,11} though, have demonstrated excessive T_H2 response capacity in individuals with active tuberculosis, whereas more current epidemiologic data¹² refute the inverse relationship between tuberculin reactivity and allergies.

Taken together, these findings emphasize the need to further explore the immunologic relationship between allergies and autoimmune diseases in the context of T-cell regulation and tolerance, the question being whether having both illnesses is associated with diminished regulatory and tolerance capacity compared with having only 1 or neither illness. We compared regulatory cytokine secretion capacities in individuals with concomitant allergies and T_H1-type autoimmunity, individuals with allergies only, and individuals with neither illness.

METHODS

Participants

An age-, sex-, and ethnicity-matched case-control study was undertaken. Case subjects and allergic controls were recruited from Northwest Asthma and Allergy Center, Seattle, WA, and healthy controls were recruited from the general population (via fliers posted at the University of Washington and by word of mouth via study investigator peers). Cases were defined as established clinic patients with diagnosed and treated allergic rhinitis who, in addition, had a physician-diagnosed T_H1-type illness determined during an interview (S.K.C.). Illnesses that met the T_H1-type criteria included type 1 diabetes mellitus (n = 3), rheumatoid arthritis (n = 6), Crohn disease (n = 3), multiple sclerosis (n = 2), thyroiditis (excluding Graves disease) (n = 3), and Sjögren syndrome (n = 3). Three cases had 2 simultaneous diagnoses. Matched controls with allergic rhinitis only and without any history or symptoms of autoimmunity were found by interview all case subjects. Matched controls with neither an autoimmune or an allergic diagnosis by history or any associated symptoms were found for all except 3 case subjects. Individuals were excluded from participation if there was any history of human immunodeficiency virus infection, tuberculosis, cancer, or undiagnosed chronic illness or, in the case of healthy controls, any long-term medication use.

Allergy Skin Testing

Skin prick testing was performed to confirm allergic status.¹³ Extracts used included saline and histamine controls, cat mix,

dust mite mix, grass mix, and mold mix (Hollister-Stier Laboratories, Spokane, Wash) and allergy clinic proprietary mixes of tree allergens (alder and birch) and weed allergens (English plantain and yellow dock).

Peripheral Blood Mononuclear Cell Culture and Cytokine Measurements

Each participant donated 15 mL of venous blood in heparinized tubes (BD Vacutainer; BD, Franklin Lakes, NJ), which was processed within 4 hours of collection. Peripheral blood mononuclear cells (PBMCs) were collected by Ficoll (ICN Pharmaceuticals, Costa Mesa, CA) density centrifugation. Cells (1×10^6) were washed with Hanks balanced salt solution (Gibco BRL, Gaithersburg, MD), counted, and cultured in 200 μ L of RPMI 1640 supplemented with L-glutamine, penicillin-streptomycin, Hepes buffer, and fetal calf serum (Gibco BRL) in 96-well plates with appropriate stimuli: lipopolysaccharide (LPS) (100 ng/mL) (Sigma-Aldrich Corp, St Louis, MO) for monocyte-derived IL-10 for 1 day; phytohemagglutinin (2 μ g/mL) (Sigma-Aldrich Corp) for T-cell-derived IL-10 for 3 days; PHA (2 μ g/mL) for IL-5, interferon γ (IFN- γ), and TGF- β for 5 days; and medium only for comparison with each stimulated culture. Supernatants from culture wells were drawn off at designated intervals and were placed in a -70°C freezer until enzyme-linked immunosorbent assay (ELISA) analysis.

Cytokine ELISAs were performed via contract by the Shared Cytokine Resource Laboratory, an independent commercial laboratory at the Fred Hutchinson Cancer Research Center (Seattle, WA). The ELISA kits were from Pierce/Endogen (Carlsbad, CA) (IL-10 and IFN- γ) and from R&D Systems (Minneapolis, MN) (TGF- β) and were used following manufacturers' instructions.

Statistical Analysis

Statistical analysis was performed using a software program (SPSS version 11.5; SPSS Inc, Chicago, IL) to compare cytokine levels between matched participants. The *a priori* assumption that cases have diminished regulatory and tolerance parameters compared with controls guided the use of 1-sided *P* values during matched analysis. To evaluate overall T-cell regulatory cytokine secretion capacity, we created an index based on an adjusted additive model of IL-10 and TGF- β levels, accounting for the difference in order of magnitude between the 2 levels. Each IL-10 and TGF- β level (*i*) was rescaled as a proportion of the highest level obtained from our population. Hence, the individual with the highest level for a particular cytokine had a value of 1, with other individuals' values rescaled to a proportion of the highest value.

$$\text{Treg}_{\text{index}}(i) = \frac{\text{IL-10}(i)}{\text{IL-10}(\text{highest})} + \frac{\text{TGF-}\beta(i)}{\text{TGF-}\beta(\text{highest})}$$

The protocols for this study were reviewed and approved by the Human Subjects Division of the University of Washington. All participants provided written informed consent.

RESULTS

Table 1 gives the characteristics of the 17 cases, 17 allergic controls, and 14 healthy controls. Thirteen cases and 9 allergic controls were taking an antihistamine, an immunosuppressant medication (including oral or nasal corticosteroids), or both. Except for 6 allergic controls, all participants who had seasonal allergic rhinitis also had perennial allergic rhinitis.

Table 2 gives mean cytokine data for each of the 3 participant groups. Statistically significant differences are noted based on matched comparison. Mean IL-10 and TGF- β levels were consistently lowest in autoimmune/allergic individuals. Allergic controls had the highest mean IL-10 levels except in day 1 unstimulated cultures, when healthy control levels were the highest. Healthy controls had the highest mean TGF- β levels. Under matched analysis, these trends were significant only between unstimulated day 3 IL-10 levels in autoimmune/allergic cases and allergic control subjects and between stimulated TGF- β levels in autoimmune/allergic cases and healthy controls. Levels of IL-5 and IFN- γ between cases and allergic or healthy controls demonstrated no clear, statistically significant trend. Overall, average IFN- γ levels of cases were lower than those of allergic controls and healthy controls. Overall, average IL-5 levels of cases were lower than those of allergic controls but higher than those of healthy controls. Under matched analysis, IL-5 levels were significantly higher in allergic controls compared with healthy controls for PHA-stimulated cultures. Unstimulated cultures had a similar trend but did not reach statistical significance.

We examined cytokine levels based on potential confounding factors, including medication use and clinical characteristics (Table 1). Among cases, LPS-stimulated IL-10 levels were significantly lower in those who were taking immunosuppressant medications compared with those who were not (732.8 vs 1,224.1 pg/mL; $P = .03$). Among allergic controls, those who were taking corticosteroid medications had higher PHA-stimulated TGF- β levels than those who were not (25,030.8 vs 15,918.3 pg/mL; $P = .02$). When subjects were

aggregated, cytokine levels did not differ significantly between subjects whether they were taking antihistamines or immunosuppressant medications or whether they had active allergic rhinitis symptoms or a concomitant asthma diagnosis, and neither were there any consistent trends (data not shown).

Figure 1 illustrates Treg indices calculated from day 1 IL-10 LPS plus day 5 TGF- β PHA-stimulated and unstimulated levels and day 3 IL-10 PHA plus day 5 TGF- β PHA-stimulated and unstimulated levels. Statistically significant associations under matched analysis are noted. Healthy subjects consistently demonstrated a statistically significant higher Treg index than autoimmune or allergic cases. Allergic controls consistently had an intermediate Treg index, although it was not significantly different from that of autoimmune or allergic case subjects and was significantly different from that of healthy control subjects only in the case of the Treg index calculated using day 3 IL-10 unstimulated levels.

DISCUSSION

The results of this study show that individuals with a T_H1 -type autoimmune disorder and allergic rhinitis have a diminished IL-10 and TGF- β cytokine production capacity, as measured by unstimulated and mitogen-stimulated PBMC culture, compared with individuals with allergic rhinitis only and with those with neither illness. The trend seen in IFN- γ and IL-5 levels, 2 prototypical T_H1 - and T_H2 -type cytokines, respectively, between allergic and healthy controls seem in part to be consistent with current theories regarding the allergic T_H2 bias. Mean stimulated and unstimulated IL-5 levels were highest in allergic controls, reflecting T_H2 skewing, and mean stimulated IFN- γ levels were highest in healthy controls, reflecting T_H1 skewing. In all 4 culture types, day 1 LPS and unstimulated and day 3 PHA and unstimulated, allergic controls consistently had the highest IL-10 levels, which on 1 occasion (day 3 unstimulated between cases and allergic controls) were statistically significant. This trend is counter to the prevailing theory that allergy

Table 1. Characteristics of the Study Participants

	Autoimmune/allergic cases (n = 17)	Allergic controls (n = 17)	Healthy controls (n = 14)
Age, mean $\pm$ SD (range), y*	45.1 $\pm$ 11.6 (22–61)	45.1 $\pm$ 11.6 (22–64)	45.9 $\pm$ 10.8 (25–61)
Sex, M/F, No. (%)	1 (6)/16 (94)	1 (6)/16 (94)	1 (7)/13 (93)
Ethnicity, No. (%)			
Asian	1 (6)	1 (6)	1 (7)
White	16 (94)	16 (94)	13 (93)
Medication, No. (%)			
Antihistamine	3 (18)	3 (18)	0
Immunosuppressant	5 (29)	4 (24)	0
Both	5 (29)	2 (12)	0
Active symptoms, No. (%)	16 (94)	11 (65)	0
Seasonal allergic rhinitis, No. (%)	6 (35)	15 (88)	0
Asthma diagnosis, No. (%)	8 (47)	6 (35)	0

* Subjects were matched on these criteria.

Table 2. Cytokine Data*

	Cytokine, mean $\pm$ SD (range), pg/mL		
	Autoimmune/allergic cases	Allergic controls	Healthy controls
IL-10			
LPS stimulated	935.1 $\pm$ 478.11 (284.90–1,829.90)	1,445.00 $\pm$ 1,249.47 (182.30–4,868.00)	1,112.52 $\pm$ 1,195.27 (371.20–5,073.00)
Day 1, unstimulated	5.45 $\pm$ 4.66 (0–17.20)	6.88 $\pm$ 13.87 (0–59.30)	10.84 $\pm$ 19.72 (0–72.90)
PHA stimulated	726.18 $\pm$ 532.19 (171.10–2,076.70)	1058.51 $\pm$ 812.91 (240.50–3,582.30)	921.79 $\pm$ 460.03 (223.40–1,969.90)
Day 3, unstimulated	5.71 $\pm$ 5.03 (0–16.00)†	11.52 $\pm$ 13.09 (0–41.6)†	10.94 $\pm$ 13.16 (2.90–52.30)
TGF- β			
PHA stimulated	17,484.31 $\pm$ 6,773.19 (8,864.90–30,367.90)‡	19,134.46 $\pm$ 7,997.01 (9,186.60–33,463.60)	23,822.55 $\pm$ 11,370.47 (10,865.90–42,950.40)‡
Unstimulated	15,710.62 $\pm$ 7,642.27 (7,807.70–30,828.10)	17,913.51 $\pm$ 10,144.26 (6,422.20–52,628.80)	20,383.08 $\pm$ 10,053.33 (8,875.40–39,378.00)
IFN- γ			
PHA stimulated	86.19 $\pm$ 171.66 (0–727.80)	292.79 $\pm$ 773.92 (0–3,277.30)	417.84 $\pm$ 956.83 (37.70–3,649.20)
Unstimulated	40.33 $\pm$ 47.94 (0–164.70)	43.68 $\pm$ 28.74 (7.10–106.60)	41.43 $\pm$ 20.17 (17.40–86.30)
IL-5			
PHA stimulated	16.19 $\pm$ 27.03 (0–72.30)	36.03 $\pm$ 43.21 (0–109.40)†	10.64 $\pm$ 11.89 (0–36.50)†
Unstimulated	24.18 $\pm$ 41.95 (0–175.80)	43.88 $\pm$ 61.58 (0–199.80)	13.19 $\pm$ 13.89 (0–37.70)

Abbreviations: IFN- γ , interferon γ ; IL-10, interleukin 10; LPS, lipopolysaccharide; PHA, phytohemagglutinin antigen; TGF- β , transforming growth factor β .

* Significant differences were based on matched analysis. The detection limit for IL-10 was 0.8 pg/mL, for TGF- β was 3.0 pg/mL, for IFN- γ was 0.3 pg/mL, and for IL-5 was 0.8 pg/mL.

† $P = .05$.

‡ $P = .02$.

results in decreased IL-10 levels.³ Why our data are contradictory is unclear and may be related to the immune-modulating medications that participants were taking at the time of recruitment.

The possibility that medication use and clinical characteristics of participants may have confounded or modified our results was refuted when we aggregated and compared subjects based on medication use and symptom characteristics. Such an analysis did not show a clear trend. The pitfall with such a comparison is that none of the healthy controls were taking antihistamines or immunosuppressant drugs. Our results, at least in comparing healthy controls with either cases or allergic controls, may simply reflect the effect of such medications on IL-10 and TGF- β production.

There is the possibility of misclassification bias, which may have reduced our ability to discern more of a gradient in regulatory cytokine secretion capacity. Atopic controls may have had a T_H1-type subclinical pathophysiology and healthy controls may have had either a T_H1- or a T_H2-type subclinical pathophysiology. One could argue that skin prick testing along with clinical history rules out possible bias from underestimating and misclassifying a T_H2-type pathophysiol-

ogy. There is no similar marker for T_H1-type aberrant physiologic processes. Such a situation is conceivable given the characteristics of these T_H1-type autoimmune illnesses, particularly with Crohn disease, thyroiditis, multiple sclerosis, and type 1 diabetes mellitus, which can all manifest preclinical forms.^{14–17} The heterogeneity of our case population's T_H1-type diagnoses may be further cause for misclassification bias or at least a diluting of an underlying gradient resulting from particular T_H1-type autoimmunity. Ideally we would have recruited a stratified sample of case subjects representing each of the main diagnoses and included so-called T_H2-type autoimmune disorders as well, such as lupus.

In addition, there are myriad potential confounding factors that may be associated with our classification of subjects and the results of their immunologic evaluation. These confounders may include the specific allergen to which subjects were sensitized and to which subjects were not matched as well as undocumented immunologically relevant clinical events, such as environmental and microbial cross-reactivity and triggers of illness. In relation to these concerns, we used a mitogen, PHA, to stimulate PBMCs. Allergens may have been more appropriate, emulating the physiologic processes

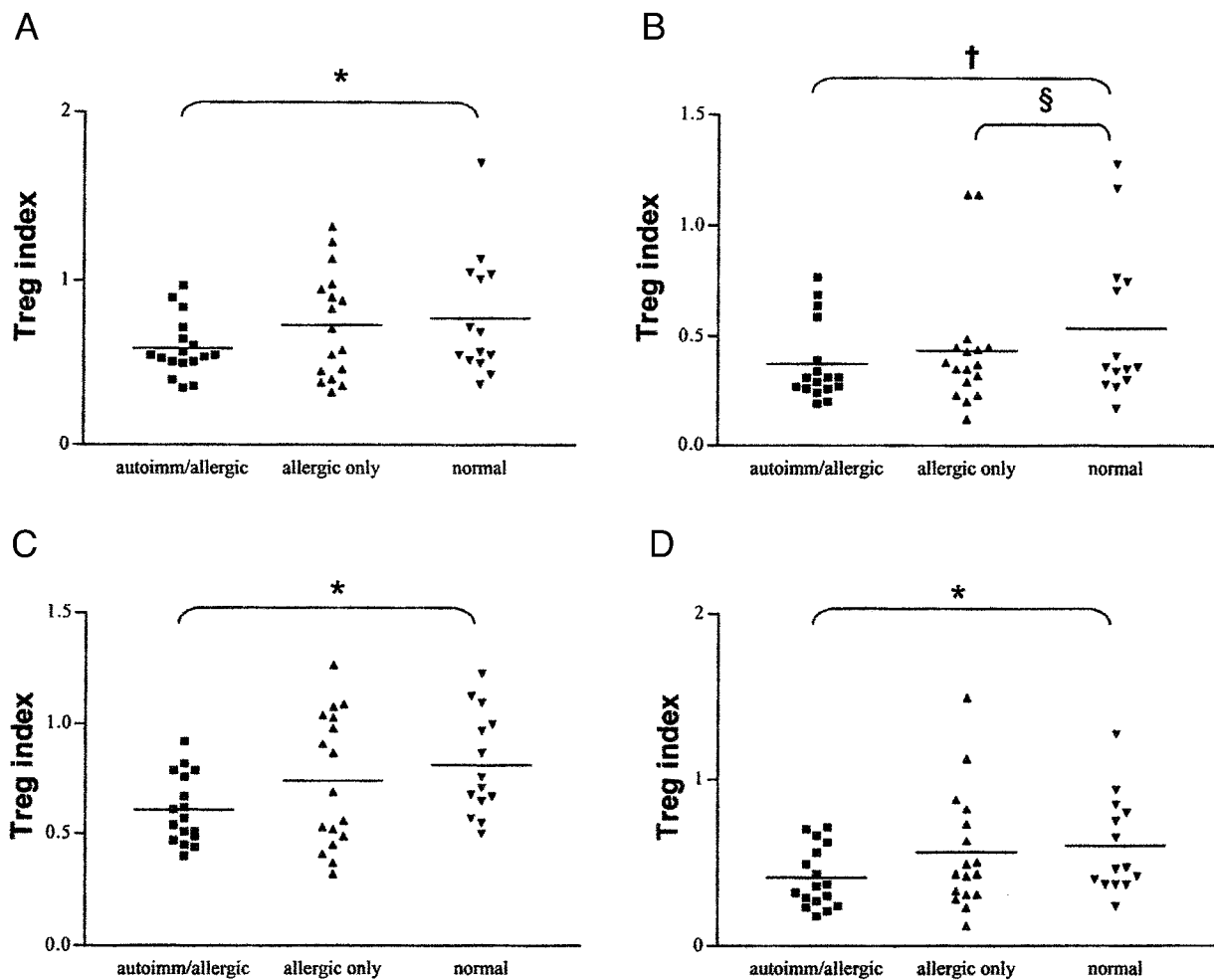


Figure 1. T-regulatory cell (Treg) indices based on results from Table 2, using an adjusted additive model (see the "Statistical Analysis" subsection of the text). A, The Treg index from day 1 lipopolysaccharide-stimulated interleukin 10 (IL-10) levels and day 5 phytohemagglutinin (PHA)-stimulated transforming growth factor β (TGF- β) levels. B, The Treg index from day 1 unstimulated IL-10 levels and day 5 unstimulated TGF- β levels. C, The Treg index from day 3 PHA-stimulated IL-10 levels and day 5 PHA-stimulated TGF- β levels. D, The Treg index from day 3 unstimulated IL-10 levels and day 5 unstimulated TGF- β levels. Asterisk indicates $P = .05$ based on matched analysis between cases and healthy controls; dagger, $P = .005$ based on matched analysis between cases and healthy controls; and section mark, $P = .05$ based on matched analysis between allergic and healthy controls; and autoimm indicates autoimmune. Horizontal bars represent arithmetic means.

within the body, but there was no way to determine which specific allergen to use in these situations. Distinguishing and matching subjects based on the clinical characteristics of their allergic rhinitis diagnosis (eg, perennial or seasonal) could have assisted in clarifying this situation.

In the absence of a more precise means of assessing T-cell regulation and tolerance, such as phenotypic and functional characterization of Treg, we elected to create a compound index summing IL-10 and TGF- β results. This index has not yet been validated. As a test for reliability, we reanalyzed the data using the arithmetic mean cytokine value as the standardizing denominator instead of the highest cytokine value obtained in our sample population. This analysis resulted in the identical trend, where our indices were consistently low-

est for cases, intermediate for allergic controls, and highest for healthy controls (data not shown). When using this method, there were statistically significant differences between allergic and healthy control unstimulated day 1 IL-10 plus day 5 TGF- β indices, case and healthy control PHA-stimulated IL-10 plus TGF- β indices, and case and allergic control unstimulated day 3 IL-10 + day 5 TGF- β indices.

Some investigations^{18,19} refute a positive association between allergy and autoimmunity, in contrast to the aforementioned studies^{7,8} in support of such a link. However, these investigations did not take into account concomitant diagnoses and related immunologic ramifications. Comparing individuals with and without simultaneous pathophysiologic processes, as in the present study, may have accentuated the

mutual, critical difference in expression of T-cell regulation and tolerance, which these previous investigations did not consider.

The significance of T-cell regulation and tolerance in concomitant allergy and T_H1-type autoimmunity that this study suggests has important implications not only for these disorders but for infectious disease immunology as well. In a mouse model of *Leishmania*, CD4⁺CD25⁺ T cells had a critical role in the development of concomitant immunity.²⁰ These Treg allowed for persistent, low-level but stable infection at the original site of inoculation that at the same time prohibited new infection elsewhere. Likewise, IL-10 has been shown to have a defining role in malaria and helminth parasitism in humans.^{21,22} Nonetheless, the parallel pathophysiology between parasites on the one hand and allergies and autoimmunity on the other and their association is still not fully clarified. It is likely that the T_H1/T_H2 differentiation pattern is a result of simultaneous co-evolution with helminthes, potent T_H2 stimulators, and T_H1-provoking pathogens, such as tuberculosis and malaria. Atopy and autoimmunity may be the clinical vestiges resulting from the failure to develop an adaptive, regulated response owing to exposure to these once pervasive, chronic stimulants of the human immune system. With improved hygiene and sanitation, as in the developed world, these infectious agents are not present to stimulate immune regulation as they do in environments where they continue to be highly prevalent, hence the observed inverse prevalence of allergy and autoimmunity between the developed and developing worlds.

To our knowledge, this is the first reported investigation of the regulatory cytokine production capacity of humans with concomitant allergic rhinitis and T_H1-type autoimmunity. Further studies should include an additional comparison group with autoimmune illness only and no evidence of atopy. Characterization and functional evaluation need to be made as well of CD4⁺CD25^{high} cells in these populations to further clarify Treg activity and capacity. The results of the present investigation of critically evaluated subjects in terms of medical history and accompanying physician diagnoses have important implications in tolerance immunology because they provide data for a potential mechanism of immune regulation in autoimmunity and allergy.

ACKNOWLEDGMENTS

We thank Leonard C. Altman, MD, Stephen Tilles, MD, and the physicians and staff of Northwest Asthma and Allergy Center and the staff of Asthma Inc for their assistance in project logistics and subject recruitment and processing; Sandy Johnston and Coralie Baker for laboratory assistance; Rick Lawler and the Shared Resource Cytokine Laboratory at the Fred Hutchinson Cancer Research Center for contracted ELISA measurements; Desiree van der Kleij, PhD, for assistance with data analysis; and Lincoln Diagnostics Inc for donated supplies.

REFERENCES

1. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. 2002;347:911–920.
2. Sakaguchi S. Naturally arising CD4⁺ regulatory T-cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol*. 2004;22:531–562.
3. Reefer AJ, Carneiro RM, Custis NJ, et al. A role for IL-10-mediated HLA-DR7-restricted T cell-dependent events in development of the modified Th2 response to cat allergen. *J Immunol*. 2004;172:2763–2772.
4. Roncarolo MG, Battaglia M, Gregori S. The role of interleukin 10 in the control of autoimmunity. *J Autoimmun*. 2003;20:269–272.
5. Vigiotta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4⁺CD25⁺ regulatory T cells in patients with multiple sclerosis. *J Exp Med*. 2004;199:971–979.
6. Ling EM, Smith T, Nguyen XD, et al. Relation of CD4⁺CD25⁺ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet*. 2004;363:608–615.
7. Kero J, Gissler M, Hemminki E, Isolauri E. Could TH1 and TH2 diseases coexist? evaluation of asthma incidence in children with coeliac disease, type 1 diabetes, or rheumatoid arthritis: a register study. *J Allergy Clin Immunol*. 2001;108:781–783.
8. Sheikh A, Smeeth L, Hubbard R. There is no evidence of an inverse relationship between Th2-mediated atopy and Th1-mediated autoimmune disorders: lack of support for the hygiene hypothesis. *J Allergy Clin Immunol*. 2003;111:131–135.
9. von Mutius E, Pearce N, Beasley R, et al. International patterns of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema. *Thorax*. 2000;55:449–453.
10. Suzuki N, Kudo K, Sano Y, Ito K. Can *Mycobacterium tuberculosis* infection prevent asthma and other allergic disorders? *Int Arch Allergy Immunol*. 2001;124:113–116.
11. Talreja J, Bhatnagar A, Jindal SK, Ganguly NK. Influence of *Mycobacterium tuberculosis* on differential activation of helper T-cells. *Clin Exp Immunol*. 2003;131:292–298.
12. Grüber C, Paul KP. Tuberculin reactivity and allergy. *Allergy*. 2002;57:277–280.
13. Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. *J Allergy Clin Immunol*. 1998;101:153–156.
14. Thjodleifsson B, Sigthorsson G, Cariglia N, et al. Subclinical intestinal inflammation: an inherited abnormality in Crohn's disease relatives? *Gastroenterology*. 2003;124:1728–1737.
15. Cooper DS. Subclinical hypothyroidism. *N Engl J Med*. 2001;345:260–265.
16. McDonnell GV, Cabrera-Gomez J, Calne DB, et al. Clinical presentation of primary progressive multiple sclerosis 10 years after the incidental finding of typical magnetic resonance imaging brain lesions: the subclinical stage of primary progressive multiple sclerosis may last 10 years. *Mult Scler*. 2003;9:204–209.
17. Knip M. Natural course of preclinical type 1 diabetes. *Horm Res*. 2002;57(suppl 1):6–11.
18. Olesen AB, Juul S, Birkebaek N, Thestrup-Pedersen K. Association between atopic dermatitis and insulin-dependent diabetes mellitus: a case-control study. *Lancet*. 2001;357:1749–1752.

-
19. Oro AS, Guarino TJ, Driver R, et al. Regulation of disease susceptibility: decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. *J Allergy Clin Immunol*. 1996;97:1402–1408.
 20. Belkaid Y, Piccirillo CA, Mendez S, et al. CD4⁺CD25⁺ regulatory T cells control *Leishmania* major persistence and immunity. *Nature*. 2002;420:502–507.
 21. Turner JD, Faulkner H, Kamgno J, et al. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J Infect Dis*. 2003;188:1768–1775.
 22. Ho M, Schollaardt T, Snape S, et al. Endogenous interleukin-10

modulates proinflammatory response in *Plasmodium falciparum* malaria. *J Infect Dis*. 1998;178:520–525

Requests for reprints should be addressed to:

Sanders K. Chai, MD, MPH

Department of Occupational and Environmental Health Sciences

School of Public Health and Community Medicine

University of Washington

Seattle, WA 98195-7234

E-mail: sanderschai@yahoo.com
