Serologic Assessment of Type 1 and Type 2 Immunity in Healthy Japanese Adults

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Abstract

We assessed the informativeness of several serologic biomarkers of immune function using serum specimens collected in the Miyazaki Cohort Study from subjects who were seronegative for anti–human T-cell lymphotrophic virus I and anti–hepatitis C virus. To broadly characterize type 1 immune status, we measured EBV antibody titers, because titer profiles associated with cellular immune suppression are well described. We also tested for three type 2 biomarkers: total serum IgE, soluble CD23, and soluble CD30. Nonreactivity to a tuberculin purified protein derivative (PPD) skin test is indicative of diminished delayed-type hypersensitivity (type 1) responsiveness in the study population due to a history of tuberculosis exposure or *Bacillus Calmette-Guérin* vaccination. We therefore evaluated the serologic markers as predictors of PPD nonreactivity using logistic regression. Subjects whose EBV antibody profiles were consistent with deficient type 1 immunity were more than thrice as likely to be PPD nonreactive as persons with "normal" antibody titers. Elevated total IgE was also strongly associated with PPD nonreactivity (odds ratio 3.4, 95% confidence interval 1.2-9.9); elevated soluble CD23 had a weaker, but positive, odds ratio, whereas soluble CD30 levels were not predictive of PPD status. Therefore, PPD nonreactivity is associated, in this population, with a pattern of serum biomarkers that is indicative of diminished type 1 and elevated type 2 immunity. We conclude that, with the exception of soluble CD30, the serologic markers are informative for the characterization of type 1/type 2 immune status using archived sera from study populations of healthy adults. (Cancer Epidemiol Biomarkers Prev 2004;13(8): 1385–91)

Introduction

The type 1/type 2 cytokine paradigm has been used to characterize immune system dysfunction in a variety of clinical conditions. In brief, type 1 cytokines include interleukin (IL)-12, IL-2, and interferon (IFN)-γ and stimulate cellular immune responses (1-3), such as the delayed-type hypersensitivity response to recall antigens and the activation of cytotoxic T lymphocytes (2). In contrast, secretion of type 2 cytokines, which include IL-4, IL-10, and IL-13, leads to the activation of humoral immunity and antibody secretion (1-3). Stimulation of the humoral immune response results in the proliferation and differentiation of B lymphocytes and in the secretion of high levels of IgE (2, 3). In immunocompetent persons, cytokine secretion is tightly regulated and balanced, whereas type 1 and/or type 2 dysregulation may occur in cases of immune deficiency (1, 2).

We evaluated the informativeness of several serologic type 1 and type 2 biomarkers in the Miyazaki Cohort Study using the study's extensive serum repository. Due to the potential effects of freeze/thaw cycles (4, 5), we did not directly measure cytokines. Instead, we chose EBV serology to broadly characterize type 1 responses, because EBV antibody patterns indicative of cellular immune dysfunction are well described (6). For type 2 biomarkers, we measured serum levels of total IgE, soluble CD23 (sCD23), and soluble CD30 (sCD30). Elevated total IgE levels indicate an activated humoral immune response and secretion of IL-4 (1, 2). CD23 is a low-affinity receptor for IgE with limited expression on naïve cells (7, 8). In response to IL-4, the receptor is up-regulated on B lymphocytes and rapidly cleaved to the soluble molecule (7, 8). The presence of sCD23 in peripheral blood thus represents activated proliferating B cells and a type 2 cytokine milieu (8, 9). Likewise, CD30, a cytokine receptor of the tumor necrosis factor superfamily (10), has virtually no constitutive expression on resting or naïve cells (11, 12). The detection of sCD30 in human sera thus reflects the presence of activated proliferating type 2 helper T lymphocytes and increased IL-4 secretion (13, 14).

The present study population experienced community exposure to tuberculosis during the postwar period or

received *Bacillus Calmette-Guérin* vaccination through a national immunization program (15). Thus, a subject's nonreactivity to dermal challenge with purified protein derivative (PPD) is indicative of deficient cellular (type 1) immunity. To assess the informativeness of EBV antibody patterns and serum levels of total IgE, sCD30, and sCD23 for characterizing immune function, we evaluated their ability to predict PPD nonreactivity. Under the assumption that the PPD nonreactivity observed in study subjects reflects type 1/type 2 dysregulation, we hypothesized that persons who were nonreactive to PPD would exhibit an EBV antibody profile indicative of depressed cellular immunity and elevated levels of each type 2 serologic marker. Because the Miyazaki Cohort Study includes a substantial proportion of persons who are carriers of human T-cell lymphotrophic virus I (HTLV-I) and/or seropositive for anti–hepatitis C virus (HCV), we restricted these analyses to subjects who were seronegative for antibodies to both viruses.

Materials and Methods

Study Population. The Miyazaki Cohort Study (16) is a prospective study of the natural history of HTLV-I and HCV, in which adult residents of two small, rural villages in southwestern Japan were followed from 1984 to 2000. HTLV-I infection is endemic in this region, and ~26% of the >2,000 participants are seropositive for anti-HTLV-I (17). One study village also has high levels of HCV infection, with a seroprevalence of ~23% (18). The subjects for the present study were drawn from the 273 participants from that village who had data on PPD reactivity and sufficient serum volume for immune marker testing. Of these 273 persons, the 152 who were seronegative for anti-HTLV-I and anti-HCV are included in the present analysis.

Data Collection. Details of data collection for the Miyazaki Cohort Study have been published elsewhere (16). In brief, all study data were obtained during free annual, government-sponsored health examinations. Collected blood specimens were stored at −30°C until use. Portions of each sample were subsequently shipped, packed in dry ice, to the Harvard School of Public Health (Boston, MA), where they were stored at −80°C until biomarker testing for the present analysis. Anti-HTLV-I and anti-HCV serostatus was determined as has been described previously (19).

Type 1 and Type 2 Biomarkers. The serologic markers were measured in blood specimens collected at the screening visit, during which the skin test for PPD reactivity was administered, using samples stored at the Harvard School of Public Health. As part of a study of viral effects on immune function, serum specimens from subjects who were seropositive for anti-HTLV-I and/or anti-HCV were tested at the same time as the seronegative samples pertinent to the present study. Each assay batch included aliquots from virus antibody-positive and antibody-negative persons, and laboratory technicians were blinded to serum antibody status. The distributions of values obtained for each serologic biomarker among the anti-HTLV-I and anti-HCV negative subjects are summarized in Table 1. The observed levels confirm that each marker was readily detectable in the archived serum specimens.

PPD Reactivity. Skin tests for tuberculin PPD responsiveness were administered to randomly selected subjects during attendance at an annual health screen between 1987 and 1991. Skin testing was conducted, and results were characterized, according to standard Japanese clinical practice, as described previously (19-21). For the present analysis, subjects' PPD test responses were categorized as PPD "reactive" (+, ++, or +++) or "nonreactive" (− or ±; ref. 19).

EBV Serology. Antibodies to EBV antigens were measured at Virolab, Inc. (Berkeley, CA), under the direction of Dr. Evelyne Lennette, using standard immunofluorescence techniques (22, 23). Each specimen was tested for IgG antibodies to EBV nuclear antigen (EBNA), viral capsid antigen (VCA), early antigen-diffuse (EA-D), and early antigen-restricted (EA-R). Antibody titers were reported as the highest of serial 2-fold dilutions to yield a positive reading on immunofluorescence. Negative titers were defined as <1:5 for anti-EBNA and both forms of anti-EA and <1:20 for anti-VCA. To confirm the specificity of the observed anti-EBNA reactions, sera were tested for reactivity to BJAB cells, which do not harbor EBV (23). Two (1.3%) of the 152 subjects in the present study showed nonspecific serum reactivity to EBNA and were removed from analyses related to EBV antibody titers.

Serum IgE. IgE testing was done for the 128 anti-HTLV-I and anti-HCV seronegative subjects with sufficient serum volume using the UniCAP Total IgE

Table 1. The distribution of values of serologic type 1 and type 2 biomarkers

Biomarker	% (*n*) Seropositive*	Mean†	Range‡
Type 1 EBV antibody titers (*n* = 152)			
Anti-EBNA	98.7 (150)	1:255	1:5-1:2,560
Anti-VCA	98.7 (150)	1:803	1:80-1:5,120
Anti-EA-R	3.2 (5)	1:61	1:20-1:160
Anti-EA-D	10.5 (16)	1:19	1:5-1:160
Type 2			
Total serum IgE, kilounits/L (*n* = 128)	99.2 (127)	390.0 ± 593.4	3.3-3,653.0
sCD23, units/mL (*n* = 104)	100.0 (104)	52.5 ± 16.9	15.5-104.0
sCD30, units/mL (*n* = 107)	100.0 (107)	47.1 ± 33.8	20.4-317.2

*Of all subjects tested for a given marker.
†Geometric mean titer for EBV antibodies in seropositive subjects or arithmetic mean ± SD for type 2 markers among subjects with levels above assay detection limits.
‡Among marker seropositive subjects.

Fluoroenzymeimmunoassay (Pharmacia and Upjohn, Kalamazoo, MI). The assays were conducted in the laboratory of Dr. Craig Lilly (Brigham and Women's Hospital, Boston, MA) according to the manufacturer's instructions. All samples were tested in duplicate, and we observed an average intraassay coefficient of variation of 6.2% among all 235 subjects tested (including subjects seropositive for anti-HTLV-I and/or anti-HCV). The assay limit of detection is 2.0 kilounits/L. One (0.8%) subject had a total IgE level below the detection limit. That person's total IgE value was omitted from the computation of mean levels (Table 1) but was included in the lowest tertile group in categorical analyses.

sCD23 and sCD30. sCD23 was measured with the Human sCD23 ELISA kit (Bender MedSystems, Vienna, Austria). Of the 152 subjects in the present analyses, 107 had sufficient specimen volume for sCD23 testing. The Human sCD30 ELISA kit (Bender MedSystems) was used to measure sCD30 in the 104 anti-HTLV-I and anti-HCV seronegative subjects with sufficient serum volume. Assays were done in duplicate according to manufacturer's instructions in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA). The average intra-assay coefficient of variation was 3.6% for sCD23 and 5.3% for sCD30 when estimated in all 217 and 224 samples tested, respectively. We included blinded repeat samples to assess the between-batch reproducibility of the assays. For sCD23, we observed a mean interassay coefficient of variation of 13.6% in six repeat tests of each of two control samples. For sCD30, the mean interassay coefficient of variation was 7.1% using the same control samples. The assay limits of detection are 10.3 units/mL for sCD23 and 0.5 units/mL for sCD30. All of the subjects tested for sCD23 and sCD30 had levels above the respective detection limits (Table 1).

Statistical Analyses. Statistical analyses were conducted using SAS statistical software (SAS Institute, Inc., Cary, NC). All P values were two tailed, and all tests of statistical significance assumed an α level of 0.05. We used logistic regression to evaluate serum EBV antibody titers, as well as levels of total IgE, sCD23, and sCD30, as predictors of PPD nonreactivity among subjects who were seronegative for anti-HTLV-I and anti-HCV. In the logistic models, a single serologic marker (such as an EBV antibody titer variable or a type 2 indicator) was included as an independent predictor, and PPD nonreactivity was entered as the dependent variable. The odds ratios (OR) from these models provided estimates of the magnitude of the association of the independent predictor with PPD nonreactivity. In addition, 95% confidence intervals (CI) were estimated around each OR to describe its precision and statistical significance (24), and P values corresponding to each OR and 95% CI were obtained from the Wald χ^2 test. We controlled for potential confounding by gender, age, and smoking history (current or past versus never) in multivariate models. We also evaluated confounding by alcohol consumption (ever versus never). However, adjustment for this factor did not notably change the effect estimates, and alcohol use was therefore not included in the final models.

Type 1 Biomarkers (EBV Antibodies) and PPD Reactivity. EBV infection in immunocompetent persons is predom-

inantly latent, with a typically stable antibody profile that includes moderate titers of anti-EBNA and anti-VCA, usually with no anti-EA detectable (6). In contrast, patients with severe clinical immune deficiencies have elevated anti-VCA and anti-EA titers and low or absent anti-EBNA titers, a pattern indicative of compromised cellular immune control of EBV (6) and thus consistent with diminished type 1 immunity. Immunocompromised persons would therefore also be expected to have relatively high ratios of anti-VCA to anti-EBNA titers. Indeed, an anti-VCA to anti-EBNA titer ratio of ≥4 was found empirically to predict clinical immune dysfunction in the extensive clinical experience of Dr. Lennette's laboratory.[9] In addition, geometric mean titer ratios of Hodgkin's and non-Hodgkin's lymphoma patients were observed to be ~6- and 14-fold higher, respectively, than those of healthy controls (23).

We assumed that, in the present study population, observed PPD nonreactivity reflects systemic type 1 dysregulation, and we therefore hypothesized that PPD nonreactive subjects would be more likely than PPD responsive subjects to have EBV antibody patterns consistent with diminished type 1 immunity. Thus, we expected that low anti-EBNA, high anti-VCA, and/or high anti-EA titers would be predictive of PPD nonreactivity (6). To test this hypothesis, we categorized anti-EBNA, anti-VCA, and anti-EA titers according to their distributions among all 150 EBV seropositive subjects. These distributions did not accommodate quantile-based categorization. We therefore created anti-EBNA and anti-VCA titer variables with three categories each by combining adjacent strata that had similar ORs for PPD nonreactivity in preliminary analyses to improve statistical precision (24). The final titer categories for anti-EBNA were ≤1:80, 1:160 to 1:320, and ≥1:640. For anti-VCA, the final groups were ≤1:320, 1:640 to 1:1,280, and ≥1:2,560. Because the prevalences of seropositivity for anti-EA-R and anti-EA-D were too low to analyze separate titer categories (Table 1), we defined subjects as seropositive for any anti-EA if the titer was >1:5 for at least one of the two forms and seronegative otherwise. We constructed separate logistic regression models to test the associations of the low (≤1:80) and high (≥1:640) categories of anti-EBNA titer, the middle (1:640-1:1280) and high (≥1:2,560) categories of anti-VCA titer, and anti-EA seropositivity with PPD nonreactivity.

To evaluate combined antibody titer patterns as predictors of PPD nonreactivity, we computed the anti-VCA to anti-EBNA titer ratio (categorized for analysis as >4.0, ≤4.0), and we defined a joint classification of anti-VCA and anti-EBNA titers. To create the latter term, the two individual titer variables were dichotomized, based on the similarity of titer-specific ORs observed in the main effects analyses, to yield a joint classification term with the following four categories: anti-VCA ≤ 1:320, anti-EBNA ≥ 1:160 (i.e., both ''normal''); anti-VCA ≤ 1:320, anti-EBNA ≤ 1:80 (''normal'' anti-VCA, ''low'' anti-EBNA); anti-VCA ≥ 1:640, anti-EBNA ≥ 1:160 (''high'' anti-VCA, ''normal'' anti-EBNA); and anti-VCA ≥ 1:640, anti-EBNA ≤ 1:80 (''high'' anti-VCA, ''low'' anti-EBNA). We used separate

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[9] E. Lennette, unpublished data.

logistic regression models to determine whether the anti-VCA to anti-EBNA titer ratio or the joint classification of anti-VCA and anti-EBNA predicted PPD nonreactivity. The prevalence of anti-EA seropositivity was too low to warrant the study of interactions of anti-EA with other EBV antibody titers.

Type 2 Biomarkers and PPD Reactivity. We hypothesized that PPD nonreactivity reflects underlying type 2 predominance and that subjects who were nonreactive to a PPD skin test would be more likely than those with positive PPD responses to have elevated levels of total IgE, sCD23, and sCD30. The data allowed tertile categorization of total IgE (≤71.0, >71.0 to 314.0, >314.0 kilounits/L), sCD23 (≤45.8, >45.8 to 58.0, >58.0 units/mL), and sCD30 (≤35.7, >35.7 to 47.9, >47.9 units/mL). We conducted logistic regression analysis of the association of the second and third tertile categories of these markers with PPD nonreactivity using separate logistic models for each type 2 biomarker. To test the two-way correlations of the type 2 biomarkers with one another, we computed a Spearman rank correlation coefficient for each pairwise comparison of the continuous total IgE, sCD23, and sCD30 variables. We assessed the statistical significance of the correlation coefficients using the one-sample t test.

Combined Prediction of PPD Reactivity by Serologic Biomarkers. To evaluate whether the serologic biomarkers were more informative in combination than individually for characterizing immune status, we constructed score-type variables to tally a subject's total number of abnormal EBV antibody titers, type 2 marker levels, and all serum type 1 and type 2 biomarker values. When creating the score variables, ''abnormal'' values were defined according to the associations with PPD nonreactivity observed for each individual marker in main effects analyses. We constructed separate logistic regression models in which each of the three score variables was entered as an independent predictor of PPD nonreactivity. We hypothesized that persons with multiple abnormal serum biomarker values would be more likely than those with fewer or with only one abnormal level to have a systemic condition of immune dysfunction, as indexed by PPD nonreactivity.

Results

In logistic regression analyses, individual EBV antibody titers showed weak to moderate associations with PPD reactivity (Table 2). We observed a higher relative risk of PPD nonreactivity among subjects with comparatively low anti-EBNA titers, with anti-VCA titers in the middle or highest categories, or with detectable anti-EA. None of the ORs were statistically significant as evidenced by the inclusion of the null value of 1.0 in the 95% CIs. However, the direction of each antibody's association with PPD nonreactivity was consistent with our hypotheses.

Analysis of the joint classification of anti-VCA and anti-EBNA titers revealed that, compared with subjects having normal titers of both antibodies (i.e., anti-VCA ≤ 1:320 and anti-EBNA ≥ 1:160), participants with a high anti-VCA and/or low anti-EBNA titer had ~3.5-fold higher risk of PPD nonreactivity after adjustment for age, gender, and smoking. The increased risk of PPD nonreactivity with the abnormal antibody combinations is again consistent with our hypothesis that patterns indicative of diminished cellular (type 1) immunity would predict PPD nonreactivity. In contrast, and contrary to expectation, an elevated ratio of anti-VCA to anti-EBNA titers (>4.0) did not predict nonreactivity to PPD (data not shown).

For the individual type 2 biomarkers, the most noteworthy predictor of PPD nonreactivity was serum total IgE (Table 3); a level in the highest tertile category (>314.0 kilounits/L) conferred a statistically significant ($P = 0.02$) >3-fold higher risk compared with levels in the lowest tertile (<71.0 kilounits/L). sCD23 levels in the

Table 2. The association of serum EBV antibody titer patterns with skin nonreactivity to PPD

Antibody Titer/Pattern	% Specified Titer Level by PPD Status		OR (95% CI) for PPD Nonreactivity, Adjusted for Age, Gender, and Smoking History
	Nonreactive ($n = 50$)	Reactive ($n = 100$)	
Anti-EBNA			
≤1:80	18.0	15.0	1.2 (0.5-3.3)
1:160-1:320	60.0	58.0	1.0
≥1:640	22.0	27.0	0.6 (0.3-1.5)
Anti-VCA			
≤1:320	22.0	34.0	1.0
1:640-1:1,280	54.0	46.0	2.2 (0.9-5.3)
≥1:2,560	24.0	20.0	2.1 (0.7-6.0)
Anti-EA			
Seronegative (<1:5)	82.0	88.0	1.0
Seropositive (≥1:5)	18.0	12.0	1.7 (0.6-4.5)
Joint classification of anti-VCA* and anti-EBNA[†]			
Anti-VCA normal, anti-EBNA normal	12.0	27.0	1.0
Anti-VCA normal, anti-EBNA low	10.0	7.0	3.7 (0.8-17.6)
Anti-VCA high, anti-EBNA normal	70.0	58.0	3.4 (1.2-9.9)
Anti-VCA high, anti-EBNA low	8.0	8.0	3.7 (0.7-18.7)

*Anti-VCA titers are ''normal'' if ≤1:320 and ''high'' if ≥1:640.
[†]Anti-EBNA titers are ''normal'' if ≥1:160 and ''low'' if ≤1:80.

Table 3. The association of serologic type 2 biomarkers with skin nonreactivity to PPD

Biomarker	% Specified Titer Level by PPD Status		OR (95% CI) for PPD Nonreactivity, Adjusted for Age, Gender, and Smoking History
	Nonreactive*	Reactive	
Total IgE (kilounits/L)	(n = 45)	(n = 83)	
≤71.0	24.4	37.4	1.0
>71.0-314.0	35.6	32.5	1.6 (0.6-4.3)
>314.0	40.0	30.1	3.4 (1.2-9.9)
sCD23 (units/mL)	(n = 37)	(n = 70)	
≤45.8	29.7	34.3	1.0
>45.8-58.0	29.7	35.7	1.0 (0.3-2.9)
>58.0	40.5	30.0	1.5 (0.5-4.2)
sCD30 (units/mL)	(n = 34)	(n = 70)	
≤35.8	32.4	34.3	1.0
>35.8-47.9	29.4	35.7	0.8 (0.3-2.2)
>47.9	38.2	30.0	1.1 (0.4-3.4)

*Column percents may not sum to 100.0 due to rounding.

highest tertile showed a weak positive association with PPD nonreactivity compared with levels in the lowest tertile. In contrast, sCD30 levels were not associated with PPD status. Nonetheless, these observations collectively suggest that PPD nonreactive subjects have elevated type 2 immunity. Levels of sCD23 and sCD30 were positively correlated with one another; the correlation coefficient was 0.4 ($P < 0.0001$) when continuous variables were compared. In contrast, total serum IgE was not correlated with sCD23 ($r = -0.04$, $P = 0.7$) and was only weakly correlated with sCD30 ($r = 0.2$, $P = 0.06$).

We expected that combined use of the biomarkers, which were all measured in the same serum sample, would improve the serologic classification of a subject's immune status by increasing the specificity of an "abnormal" characterization over that obtained with a single marker value. As predicted, the relative risk of nonreactivity to PPD increased with increasing numbers of abnormal marker values, and subjects with at least four abnormal values (and thus evidence for concomitant type 1 and type 2 dysregulation) had a notably higher prevalence of PPD nonreactivity than those with only one abnormal biomarker level (data not shown). Thus, the biomarkers in combination were informative indicators of immune function among the subjects who were seronegative for anti-HTLV-I and anti-HCV. However, in subsequent analyses that included subjects who were seropositive for anti-HTLV-I or anti-HCV, the score variables were not more informative than the individual biomarkers for characterizing the type 1 and type 2 status of virus-seropositive subjects (data not shown). We concluded that the biomarkers in combination did not markedly enhance the characterization of immune function in this population.

Discussion

We undertook this analysis to identify biomarkers that could be used as alternatives to PPD skin testing to broadly characterize type 1 and type 2 immune status in this study population. Of the markers tested, total IgE had the strongest individual association with PPD status. Subjects with the jointly classified pattern of high anti-VCA and low anti-EBNA titers also had a moderately large relative risk of PPD nonreactivity. Serum sCD23 was not strongly associated with PPD nonreactivity, nor were individual EBV antibody titers, but the ORs estimated for these markers were nonetheless consistent with the hypothesis that PPD nonreactivity reflects both type 1 and type 2 dysregulation. Serum sCD30 did not predict PPD status and therefore does not seem to be a sensitive marker of type 2 dysregulation in this study population.

Our evaluation of anti-EBNA may have been limited by the lack of information on titers of antibodies to specific components of the EBNA complex (i.e., EBNA-1 and EBNA-2). Elevated anti-EBNA-2 and diminished anti-EBNA-1 titers, and in particular a ratio of EBNA-1 to EBNA-2 titers of <1, have been observed in cases of chronic EBV infection and are considered indicative of defective immune control of EBV latency (23, 25). Thus, measurement of anti-EBNA-1 and anti-EBNA-2 titers and/or their titer ratio may have been more informative for characterizing immune status than the single titer of antibodies to the EBNA complex.

Evaluation of the joint classification of anti-EBNA and anti-VCA titers yielded notably stronger ORs than those estimated for individual anti-EBNA and anti-VCA titer variables. One reason for these stronger associations is likely to be that the reference group for the combined variable comprised only subjects with normal titers for both antibodies, whereas, in the main effects analysis of either individual antibody, the comparison group included some subjects with abnormal titers of the other antibody. Therefore, the relative risks observed for the three abnormal titer patterns in the joint classification likely reflect more closely the true association of these abnormal titers with PPD nonreactivity.

To our knowledge, this is the first study in which PPD skin reactivity has been measured concurrently with serum EBV antibody titers and levels of all three type 2 biomarkers in a population of healthy adults. Several studies have investigated whether *Bacillus Calmette-Guérin* vaccination predicts lower total IgE levels and/or is protective against atopic disease, with inconsistent results (26). The majority of our study subjects were likely to have been exposed naturally rather than iatrogenically to mycobacteria (15). Therefore, we do not attribute the observed inverse association of PPD reactivity with total IgE to protection from atopic disease by *Bacillus Calmette-Guérin* immunization. One previous

report documented inverse correlations between positive PPD reactivity and a variety of atopic symptoms among *Bacillus Calmette-Guérin*–immunized 12- and 13-year-old Japanese school children (27). PPD nonresponders had higher total serum IgE levels than subjects with positive PPD responses in that study, an observation consistent with the current findings. Several cytokine levels were also measured in the pediatric study, and PPD nonreactive children had significantly higher serum levels of the type 2 cytokines IL-4, IL-10, and IL-13 (27); in contrast, levels of IFN-γ, a type 1 cytokine, were significantly lower among children who were nonreactive to PPD. Collectively, these data indicate that PPD nonreactivity is associated with diminished type 1 and increased type 2 immune marker levels and that total IgE levels are informative for characterizing type 2 status.

In the present study population, serum levels of sCD23 were less strongly associated, and levels of sCD30 were unassociated, with PPD nonreactivity. These findings were contrary to our expectation, because both sCD23 and sCD30 have been used to empirically detect elevated type 2 immune responses in a variety of contexts (9, 14). For each of these two markers, the means and ranges of values we observed were similar to the expected values cited in the assay kit inserts, which were obtained from tests done by the manufacturer on sera from blood donors. Therefore, it does not seem that assay insensitivity contributed to the weaker associations of these two biomarkers with PPD nonreactivity. We also observed a significant moderate correlation of sCD23 with sCD30 similar to that reported by Schönermarck et al. (28). In addition, a lack of correlation between total IgE and sCD23 has been described previously (29-31), as has a weak positive correlation between total IgE and sCD30 (32, 33). The differences between mean values within each tertile category of sCD23 and sCD30 were small, as evidenced in part by the narrow range of values that defines the middle tertile category of each variable. It is plausible that the small differences in the mean values correspond to relatively small differences in prevalence of PPD nonreactivity. In addition, many subjects with insufficient serum volume were omitted from the sCD23 and sCD30 analyses. Thus, with reduced sample sizes, we had low statistical power to detect significant small associations of tertile category of sCD23 or sCD30 with PPD nonreactivity.

Misclassification of PPD status or the serologic immune function markers could have affected the observed associations. Some subjects within the PPD nonreactive group may never have been exposed to mycobacteria and thus had healthy immune function and, presumably, normal levels of the serologic biomarkers. However, the number of such subjects is likely to be very small, because the Japanese Ministry of Health and Welfare has a longstanding program of skin testing and immunization of PPD nonreactive citizens with *Bacillus Calmette-Guérin* (15, 27). With regard to serum marker misclassification, we did not observe evidence of systematic measurement error in any of our biomarker assays, with acceptable coefficients of variation within and between test batches. Furthermore, the laboratory assays were done by experienced technicians who were blinded to PPD status. Thus, any misclassification of serologic marker levels is expected to be small and non-

differential with respect to PPD reactivity. Nonetheless, such nondifferential misclassification would further reduce statistical power and may therefore explain in part the lack of association we observed for sCD23 and sCD30 with PPD nonreactivity, because true associations between these variables were likely to be small.

In conclusion, we have showed an association between concurrent measures of several serologic type 1 and type 2 biomarkers and PPD nonreactivity in healthy Japanese adults. PPD nonreactivity was predicted consistently, if not always strongly, by EBV antibody titers indicative of deficient cellular immune control of EBV as well as by elevated levels of total serum IgE and sCD23. These findings suggest that the subclinical immune dysfunction observed as nonreactivity to PPD is characterized by both type 1 and type 2 immune dysregulation. We conclude that, with the exception of sCD30, the serologic markers evaluated are informative for broadly characterizing general immune competency and type 1/type 2 status using archived sera from populations of healthy Japanese adults and likely among healthy adults of other ethnicities as well.

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