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12. Peptide library synthesis was as described (*10*). Individual PDZ domains were expressed and purified as GST fusion proteins: murine hDlg PDZ-1 (186–282), PDZ-2 (281–377), PDZ-3 (428–518), and PDZ-1/2 (281–518); murine PTPbas PDZ-3 (1351–1445) and PDZ-5 (1758–1848); murine Tiam-1 PDZ; human LIN-2 PDZ (422–507); human erythroid p55 PDZ (1–164); and human AF-6 PDZ (983–1102). Glutathione beads (50 to 60 μl) saturated with GST-PDZ proteins were mixed with the peptide library (1 mg) in 300 μl of TSN buffer [40 mM triethylamine (pH 7.6), 150 mM NaCl, and 0.01% NP-40] containing bovine serum albumin (BSA, 1 mg/ml) and 1 mM dithiothreitol (DTT). After 45 min of constant shaking at 4°C, the beads were washed with TSN buffer. The peptides retained were eluted with 30% acetic acid, lyophilized, resuspended in distilled water, and sequenced on a Bio-Applied 477A sequencer.
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The Inverse Association Between Tuberculin Responses and Atopic Disorder

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Human immune responses are heterogeneous and may involve antagonism between T helper (T_H) lymphocyte subsets and their cytokines. Atopy is characterized by immediate immunoglobulin E (IgE)–mediated hypersensitivity to agents such as dust mites and pollen, and it underlies the increasingly prevalent disorder asthma. Among Japanese schoolchildren, there was a strong inverse association between delayed hypersensitivity to *Mycobacterium tuberculosis* and atopy. Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels, and cytokine profiles biased toward T_H1 type. Exposure and response to *M. tuberculosis* may, by modification of immune profiles, inhibit atopic disorder.

Atopy is a state of allergic response, mediated by IgE, to largely innocuous, common environmental antigens (allergens) such as those derived from house dust mites and plant pollens (*1*); it underlies the clinical diseases of asthma, hay fever, and eczema (*2*). Atopy can be recognized by allergen-specific IgE in serum or by immediate-type hypersensitivity reactions to allergens upon intradermal skin testing. Heterogeneous genetic and environmental factors interact in the development of atopy (*3*); a set of cytokines—interleukin-4 (IL-4), IL-10, and IL-13 derived from the T_H2 subset of T lymphocytes—is central in mediating IgE production and the development of immediate hypersensitivity (*4*).

In recent decades there has been an increase in severity, and probably in prevalence, of atopic disorders in developed countries (*5*). Studies on migrants from developing to developed countries support the importance of etiological environmental changes associated with "Westernization" (*6*). The nature of these environmental changes is obscure, but speculation has focused on increased air pollution or other toxins in the environment, increased indoor exposure to dust mite antigens in less ventilated modern homes, and dietary changes (*7*). One factor temporally associated with the rise of atopy is the decline of many infectious diseases in developed countries as the result of improved living standards and immunization programs (*8*). Data on the risk of atopy according to sibship size and birth order (*9*) also support the possibility that diminished exposure to infection might, in some way, promote atopic responses. Childhood respiratory infections that might strongly modify the developing immune system, both systemically and within the lung, include measles, whooping cough, and tuberculosis. Some of these infections cultivate a T_H1 immunological environment with IL-12, interferon-γ (IFN-γ), and tumor necrosis factor (TNF) as predominant cytokines (*10*); because these cytokines inhibit T_H2 cytokine functions (*11*), the absence of such infections might release T_H2 immune mechanisms and thus promote atopic disorder.

In the case of tuberculosis, an important marker of T_H1-mediated acquired immunity (not synonymous with protection) is the development of delayed-type hypersensitivity. This can be tested by observing the reaction, after 48 hours, to the intradermal injection of tuberculin protein (*12*). There is likely a "J-shaped" relation between the degree of delayed hypersensitivity and the risk of tuberculous disease, in which people with moderate hypersensitivity are at least risk (*13*).

To test for clinical evidence of antagonism between delayed hypersensitivity to tuberculin and immediate atopic responses, we conducted an epidemiologic survey in a county of the Wakayama prefecture in southern Honshu, Japan, where there has been a long-established program of tuberculin testing and immunization with attenuated bovine *M. tuberculosis* vaccine [bacillus Calmette-Guérin (BCG)] after birth and at 6 and 12 years of age (*14*). From a population of approximately 1000 12- to 13-year-old schoolchildren attending the 18 junior high schools of the county in 1995, we studied 867 children with complete retrospective records of their tuberculin responses. We administered a

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questionnaire documenting atopic symptoms and social and environmental variables, and we also measured IgE serum levels and T_H1 and T_H2 cytokine profiles (*15*); these data were analyzed in relation to the record of tuberculin responses.

There was a bimodal distribution of delayed-type hypersensitivity responses to tuberculin upon skin testing (Fig. 1A). Positive tuberculin tests (≥10 mm skin induration) correspond to response to *M. tuberculosis*; negative tests include fully negative reactions as well as intermediate reactions (5 to 9 mm) that generally reflect responses to nontuberculous environmental mycobacteria or to BCG (*16*). Positive tuberculin responses were recorded in 3% of the children at 3 months of age, in 33.2% at 6 years, and in 58.0% at 12 years. In many children, the tuberculin status changed, to either positive or negative, between the ages of 6 and 12 years (Table 1, groups 2 and 4). None of the children suffered clinical tuberculous disease at any stage, including 24 with florid tuberculin responses (>40 mm skin induration) who underwent full clinical and radiographic assessment for the disease.

Of all the children studied, 36% manifested atopic symptoms at some time. A strong inverse association was found between positive tuberculin responses at both 6 and 12 years of age and a range of atopic characteristics, including symptoms at any age and IgE levels and T_H2 cytokine profiles assayed at 12 years of age (Fig. 1B and Tables 1 and 2). In positive tuberculin responders, the rate of current atopic symptoms was one-third the rate in negative responders. Asthmatic symptoms were one-half to one-third as likely in positive tuberculin responders as in negative responders (Table 2). Moreover, remission of atopic symptoms between 7 and 12 years of age was six to nine times as likely in positive tuberculin responders. Serum IgE levels, both total and allergen-specific, were also lower in the positive tuberculin responders. The geometric mean for total serum IgE level was 112

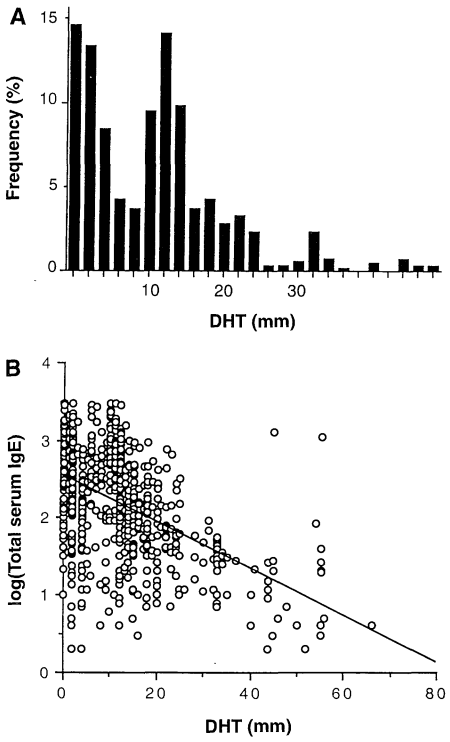

Fig. 1. Delayed hypersensitivity to tuberculin (DHT, in millimeters) and relation to serum IgE. (**A**) Histogram showing bimodal distribution of responses to tuberculin, assayed as DHT at 12 years of age in 867 Japanese schoolchildren. (**B**) Plot of log(total serum IgE) versus DHT in the same children ($r = -0.492$, $P < 0.001$).

Table 1. History of infectious diseases, atopic symptoms, IgE levels, and cytokine profiles in subjects grouped by tuberculin reactivity. ASE, allergen-specific IgE; UD, undetectable.

Measurement	Group 1 (n = 290)	Group 2 (n = 289)	Group 3 (n = 213)	Group 4 (n = 75)	Total (n = 867)
Tuberculin response					
At 6 years	−	−	+	+	
At 12 years	−	+	+	−	
Positive antiviral immunity (%)					
Measles (history + vaccine)	83.4	87.2	84.5	81.3	84.3
Chicken pox (history + vaccine)	86.9	82.3	82.2	82.7	83.9
Mumps (history + vaccine)	62.8	60.9	60.1	57.3	61.0
Number with IgE to *Ascaris*	2	2	2	1	7
Symptoms (%)					
Atopy (past + present)	46.8	33.9‡‡	25.8‡‡	38.7	36.6
Atopy (present)	32.1	7.9‡‡‡	9.8‡‡‡	30.7	18.5
Asthma (past + present)	13.4	4.1‡‡	3.7‡‡	6.8	7.4
Rhinitis (past + present)	16.2	4.8‡‡	8.6‡	14.6	10.4
Eczema (past + present)	22.7	12.8‡‡	12.2‡‡	16.0	16.2
Geometric mean IgE (IU/ml)	208	149**	98***	178	154
Positive ASE (%)	55.8	43.9‡‡	41.8‡‡	53.3	48.2
Atopic (high IgE or positive ASE) (%)	65.5	54.0‡‡	49.2‡‡	61.3	57.3
Median cytokine level (pg/ml)					
IL-4	1.88	0.96†	0.92†	1.66	1.22 (10.2-UD)§
IL-13	18.3	10.2†††	7.8†††	19.1	14.2 (45.6-UD)
IL-10	5.9	3.1††	2.9††	5.9	3.9 (10.2-UD)
IL-12	UD	UD	UD	UD	UD
IFN-γ	7.8	11.0††	13.2††	6.4	10.5 (23.2-UD)
Positive family history within three generations (%)	54.1	49.8	49.8	48.0	51.0
Mean BMI	21.1	22.0	21.9	21.2	21.6

**$P < 0.01$, ***$P < 0.001$ on the basis of Student's test. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ on the basis of a median test. ‡$P < 0.05$, ‡‡$P < 0.01$, ‡‡‡$P < 0.001$ on the basis of χ^2 against group 1, respectively. §Maximum-minimum values.

Table 2. Odds ratios for atopy and for occurrence and remission of atopic symptoms in positive versus negative tuberculin responders by age. Multiple logistic analysis was conducted with the SPSSX package, version 2.2. In all models, allowance was made for dichotomized variables including sex, life-style, nutritional status, environmental factors, and family history. Only significant values are shown.

Tuberculin response	Odds ratio		
	Atopy	Atopic symptoms	
		Occurrence	Remission
Conversion to positive up to 6 years of age	0.50 (0.29 to 0.83)*	Asthma: 0.31 (0.22 to 0.45)* Eczema: 0.50 (0.33 to 0.91)*	Asthma: 8.2 (6.0 to 9.8)** Eczema: 1.6 (1.0 to 2.2)*
Conversion to positive between 6 and 12 years of age	0.43 (0.25 to 0.83)**	Asthma: 0.42 (0.24 to 0.56)*	Asthma: 6.0 (2.8 to 10.3)*** Eczema: 6.7 (4.8 to 11.4)*** Rhinitis: 9.0 (6.2 to 14.2)***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

IU/ml for children who had a positive tuberculin response at any time, whereas it was 194 IU/ml for children whose responses were always negative. A plot of the logarithm of total serum IgE against the diameter of tuberculin response shows an inverse linear relation, $r = -0.492$ (Fig. 1B). Positive tuberculin responders had significantly lower levels of T_H2 cytokines (IL-4, IL-10, and IL-13) and higher levels of the T_H1 cytokine IFN-γ.

In tests for confounding variables (*15*), we found no differences in life-style, environmental factors, or nutritional status between the positive and negative tuberculin responders; estimated allergen exposure was similar among the groups with respect to pet animal exposure, character and ventilation of homes, and residence in a rural area. Exposure to helminths, which can promote high IgE levels, was minimal in the population; only 7 of the 867 children showed IgE to *Ascaris lumbricoides*. Similar numbers of positive and negative tuberculin responders reported atopy in any sib, parent, or grandparent (~50%) or had chest radiograph reports of tuberculosis in the same relatives at any time (~13%).

Several lines of evidence suggest that a causal link between tuberculin response and atopy is more likely than fixed determination of both atopy and diminished tuberculin responses by a genetic factor or factors. Our data show that tuberculin responses change, from positive to negative and vice versa, in many children between 6 and 12 years of age (groups 2 and 4 in Table 1). A marked decline in the incidence of positive tuberculin responses in the Wakayama region over a very short genetic interval—95% in 1965, 85% in 1975, 60% in 1985, and 58% in our survey—was accompanied by a decline in infectious clinical cases of tuberculosis from 154.4 per 100,000 in 1974 to 52.1 per 100,000 in 1994 (*17*). Experimental animal data show antigen-independent, reciprocal inhibition of either T_H1 or T_H2 immunity by infectious agents that strongly promote T_H1 responses [such as mycobacteria (*18*)] or T_H2 responses [such as schistosomes (*19*)]. The data support the hypothesis that a decline in infection, in this instance tuberculosis, is a factor underlying the rising severity and prevalence of atopic disorders in recent decades in developed countries. These data are also consistent with the idea that atopic responses are limited by T_H1 immune mechanisms.

Epidemiological data from Guinea-Bissau show that a history of childhood measles infection around the time of an epidemic was associated with a 50% decrease in the rate of positive atopic skin tests (*20*). In our study, we found no relation between a history of measles infection and atopy. However, there are important population and environmental differences between Wakayama and Guinea-Bissau; also, the Wakayama region has had an established program of measles immunization, with an uptake of 60% or more, and there had been no measles epidemic relevant to our study. It is likely that a set of specific infections that strongly promote T_H1 immunity has the potential to inhibit atopic disorder by the repression of T_H2 immunity. We believe that the role of such an infection in repressing atopy depends on a number of factors, including its timing, anatomical site, dose, and protractedness; exposure to other infections; and host characteristics such as genetic variables and nutritional status (*21*). Prospective and experimental studies are needed to investigate the action of *M. tuberculosis* and other microorganisms, through natural infection or immunization schedules, in deviating immunity away from atopy.

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