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An epidemic of tuberculosis with a high rate of tuberculin anergy among a population previously unexposed to tuberculosis, the Yanomami Indians of the Brazilian Amazon

(antimycobacterial antibodies/tuberculin skin test/genetic selection/cell-mediated immunity)

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ABSTRACT **A survey of an emerging tuberculosis epidemic among the Yanomami Indians of the Amazonian rain forest provided a unique opportunity to study the impact of tuberculosis on a population isolated from contact with the tubercle bacillus for millennia until the mid-1960s. Within the Yanomami population, an extraordinary high prevalence of active tuberculosis (6.4% of 625 individuals clinically examined) was observed, indicating a high susceptibility to disease, even among bacille Calmette–Guérin-vaccinated individuals. Observational studies on cell-mediated and humoral immune responses of the Yanomami Indians compared with contemporary residents of the region suggest profound differences in immunological responsiveness to *Mycobacterium tuberculosis* infection. Among the Yanomami, a very high prevalence of tuberculin skin test anergy was found. Of patients with active tuberculosis, 46% had purified protein derivative of tuberculosis reactions <10 mm; similarly 58% of recent bacillus Calmette–Guérin vaccines exhibited skin test reactions <5 mm. The Yanomami also had higher titers of antibodies against *M. tuberculosis* glycolipid antigens (>70%) than the control subjects comprised of Brazilians of European descent (14%). The antibodies were mostly of the IgM isotype. Among the tuberculosis patients who also produced IgG antibodies, the titers of IgG4 were significantly higher among the Yanomami than in the control population. Although it was not possible to analyze T-cell responses or patterns of lymphokine production *in vitro* because of the remoteness of the villages from laboratory facilities, the results suggest that the first encounter of the Yanomami Indian population with tuberculosis engenders a diminished cell-mediated immune response and an increased production antibody responses, relative to other populations with extensive previous contact with the pathogen. These findings suggest that tuberculosis may represent a powerful selective pressure on human evolution that over centuries has shaped the nature of human immune responses to infection.**

The Brazilian Yanomami Indians comprise about 9,400 individuals scattered throughout an area of 9.4 million hectares of the remote Amazonian rain forest of Brazil. This population remained largely isolated for millennia and largely unknown until the first decades of the 20th century (1). The first long-term contacts with individuals of European descent occurred in the mid-1960s on the periphery of their lands. Discovery of gold in the 1980s attracted 30,000–40,000 miners to the center of the indigenous territory with profound consequences for the Yanomami population. During the past three decades of contact with the outsiders, tuberculosis was introduced in the Yanomami population, as far as is known for the first time. The first reported case of tuberculosis among the Indians was dated to 1965 (2), and a small number of cases were noted in the 1970s. By the 1980s, the infection spread throughout the area and has become epidemic. According to field reports (2) the epidemic is characterized by severe disease and high mortality rates. Chest x-rays performed on bacteriologically confirmed tuberculosis patients showed extensive lobar infiltrations coincident with observed symptoms of acute respiratory disease. During the course of a medical mission†† on the Yanomami reservation to assess the importance of tuberculosis and to gather data necessary to implement adequate preventive measures to control the epidemic, a unique opportunity was provided to survey tuberculosis in a population previously unexposed to the disease. Although laboratory facilities were not available for detailed immunologic studies, it was possible to carry out an observational study of the prevalence and severity of tuberculous disease, tuberculin skin test responsiveness, and production of antibodies. The results indicate that the Yanomami are at the beginning of a severe epidemic of tuberculosis, a circumstance not previously studied systematically. They further suggest that within a previously naive population, tuberculosis engenders an unusual disease pattern, with exceptionally high rates of prevalence, tuberculin unresponsiveness, and high titers of antibody. The findings to be described suggest that tuberculosis may have been an important selective factor in shaping immune responses to infections in human populations that have long been exposed to the pathogen§§.

METHODS

Clinical and Bacteriological Analysis. Since 1990 a trained nurse had been diagnosing tuberculosis cases among the Yanomami of the visited sector based on smear results and correlative clinical symptoms and prescribing specific treat-

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Abbreviations: BCG, bacille Calmette–Guérin; PPD, purified protein derivative of tuberculosis; DAT, diacyl-trehalose.

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ments. During our visit, every present individual of this sector, including a total of 625 persons residing in five villages, was examined. In the general clinical examination, specific attention was given to symptoms and signs of respiratory ailment, adenopathies, and skin lesions. Sputum samples were collected from 122 individuals with respiratory symptoms, digested, and decontaminated, and smears were stained by Kinyoun's method (3). From each specimen, three Lowënstein–Jensen slants were inoculated, and isolates were identified by growth characteristics, colony morphology, reaction to niacin, conventional biochemical methods (3, 4), and molecular analysis with a *Mycobacterium tuberculosis* probe (Gen-Probe, San Diego). *M. tuberculosis*-positive cultures were evaluated for drug resistance by using the proportion method on Middlebrook 7H11 medium (4).

Nine *M. tuberculosis* isolates were analyzed by phage typing with bacteriophages Ag1, DS6A, GS4E, BK1, BG1, PH, D3414, DNA III, Sedge, and Legendre (5) as described (6). DNA restriction fragment length polymorphism analysis was performed on nine *M. tuberculosis* isolates as described (7). Briefly, chromosomal DNA was digested with restriction enzyme *Pvu*II, and a 860-bp PCR-amplified fragment of *IS6110* was used as DNA probe.

Serology. Peripheral blood samples were collected for serological studies from 589 individuals, excluding newborns and very young infants. All sera were assayed by a previously described ELISA (8, 9) for IgM and IgG antibodies reactive to diacyl-trehalose (DAT) and PGL-Tb1 antigens of *M. tuberculosis*. Within the patient population, titers of IgG subclasses antibodies were evaluated as described (10). To assess the possibility of an acquired immunodeficiency, such as recent measles or HIV infection, associated with a negative tuberculin skin test, 87 serum samples chosen among the tuberculin anergic responders that had been bacille Calmette–Guérin (BCG)-vaccinated or presented active tuberculosis diseases, were tested for measles (Enzygnos, Anti-Masern-virus/IgM, Behring, Marburg, Germany) and HIV gp160 and p24 antibodies. In addition, randomly chosen samples were tested for evidence of other infectious diseases that may indicate the degree of contact with outside populations, such as herpes simplex virus type 1 and type 2 (11), syphilis, and toxoplasmosis.

Skin Tests. Tuberculin skin tests were performed on 556 individuals by using two tuberculin units of purified protein derivative of tuberculosis (PPD) RT-23 (or five bioequivalents of PPD-S) supplied by the Copenhagen World Health Organization reference laboratory (Denmark) and in current use by the Brazilian Health Services. The preparation was stored between 4° and 8°C. Intradermal infections of 0.1-ml solution were administered by a trained nurse into the volar surface of the right forearm. The mean diameter of induration was measured twice, by two independent individuals, by using the "ballpoint" method (12) 72 hr after inoculation. About 3 yr before our study, a medical team visiting this sector had diagnosed several cases of tuberculosis, prescribed appropriate treatment, and performed BCG-vaccination (strain Moreau, Ataulpho de Paiva Foundation, Rio de Janeiro, Brazil) on the majority of the population. During our visit, BCG scars were sought and recorded, as were scars clearly resulting from fistulous lymph nodes. Children under 3 years of age, born after the previous visit, were BCG-vaccinated. Radiological studies were not performed during our visit because of difficult access. Specific treatment was initiated in all newly identified cases of tuberculosis disease, following the Brazilian tuberculosis recommendations (13).

RESULTS

Epidemiology of Tuberculosis Among the Yanomami Indians. In the course of a medical mission to assess the relative importance of tuberculosis among the Yanomami people and to gather data necessary to implement adequate control measures, it was possible to compile information on the response of this unique naive population to its first contact with tuberculosis. We examined all 625 inhabitants of one sector of the Brazilian Amazonian reservation representing approximately 6.6% of the total Yanomami people in Brazil. Of the 625 individuals clinically examined, 26% (162) had manifestations of respiratory disease, and 11% had acute and subacute forms of illness with cervical adenopathy (Table 1). Sputum samples were collected from 122 individuals with respiratory symptoms, and *M. tuberculosis* was identified in 13 by standard smear and culture techniques. There also were five presumed cases of primary tuberculosis in infants, defined by PPD reactions >10 mm in nonvaccinated children under 5 years of age with pulmonary symptoms, and seven cases of prominent cervical and/or submandibular lymph nodes observed in nonvaccinated individuals with skin tests >10 mm. The incidence of pulmonary and extrapulmonary tuberculosis was 25 new cases per 625 individuals or a rate of 4%, and the incidence of smear positive tuberculosis was 2% (13 of 625). In addition, 15 smear-positive patients recently had been diagnosed by the local nurse and were under treatment. Thus, for the first 6 months of 1992, the total number of tuberculosis cases in this population was 40 in 625 individuals, representing a prevalence rate of 6.4%. The prevalence of smear positive disease was 4.5%. Complete mortality rates are not available, although four deaths caused by tuberculosis were recorded among our study population during a 6-month period, representing an annual mortality rate of at least 1.28% (1,280 per 100,000).

Previous BCG Vaccination. Although precise information on BCG immunization was not available, BCG scars were found in 76% (475 of 625) of the total population. Although very few children under 3 years of age had been vaccinated, over 80% of the population above 4 years of age had BCG scars, consistent with field reports that the majority of the population had been vaccinated approximately 3 years before the present study. Among the 28 bacteriologically confirmed cases (13 new cases and 15 patients under treatment), 82% had received BCG. These results reveal unusually high prevalence and incidence rates of tuberculosis among BCG-vaccinated individuals within this population.

Bacteriological Studies. Detailed bacteriological studies were carried out on nine of the 13 *M. tuberculosis* isolates from 122 sputum cultures. All isolates were sensitive to isoniazid, rifampicin, ethambutol, and pyrazinamide used for the treatment. Restriction fragment length polymorphism analysis by using *IS6110* as molecular probe showed two different patterns. The major pattern of nine bands was found in seven isolates, and an additional pattern of 10 bands was present in two isolates. Phage typing analysis showed a profile distribution (67% AX, 22% A, and 11% I) very similar to the one found to be endemic elsewhere in Amazonia State (S. Clavel-Sères, personal communication), and previously described in Portugal (6). Although the possibility cannot be excluded that other strains might be present, the data suggest that two strains

Table 1. Clinical and bacteriological results among the Yanomami total population

Total Yanomami population in Brazilian Amazonia	9,400
Study population	625
Cases of tuberculosis (all forms)	40
Active pulmonary tuberculosis	28
Lymphnode tuberculosis	7
Primary tuberculosis	5
Estimated prevalence	6.4%
Estimated incidence	4%

originally introduced into the Amazonas region from Portugal served as source for the epidemic among the Yanomami population.

Immunological Responses to *M. tuberculosis* Infection in BCG-Immunized Individuals. Tuberculin skin tests were administered to 556 individuals, and the distribution of indurated PPD responses is presented in Fig. 1. Our data indicate strikingly low skin test responsiveness among the Yanomami people, whether BCG vaccinated or unvaccinated; even among individuals with positive reactions, the mean diameters of induration were lower than in the concurrent control population (Fig. 1). A group of military recruits also was tested with the same tuberculin PPD to validate the skin test reagent and readings. Again, striking differences in the tuberculin skin test reactivity of the Yanomami population were seen in comparison with the military recruits. First, only 42% of BCG-vaccinated Yanomami people exhibited skin reactions >5 mm. Twelve percent of BCG-vaccinated Yanomami people exhibited intermediate skin reactions (5–9 mm), and 58% had <5 mm of induration. Thus, BCG vaccinations did not enhance the proportion of low-grade PPD reactions (5–9 mm), commonly observed after BCG immunization or exposure to environmental mycobacteria in other populations (14). In contrast, of the control population of military recruits with BCG scars, 73% exhibited reactions >5 mm (54.5% = 5–9 mm), which is consistent with previously reported data in other BCG-vaccinated populations elsewhere. The difference is even more surprising given that the time elapsed between BCG vaccination and the tuberculin skin test measurement was an average of 18 years for the recruits, but only 3 years for the Yanomami. In particular, reactions of >10 mm were found in only 27% of the total Yanomami population, and in only 54% of the bacteriologically confirmed cases of tuberculosis. Tuberculin reactivity of the control population of army recruits, where no cases of active tuberculosis were found, showed a similar frequency of reactivity (43% >5 mm, 21% >10 mm) as if the tuberculosis epidemic among the Yanomami did not engender a heightened skin test response. Comparisons between a tuberculosis patient population of Brazilians of European descent (80% >10 mm) and the Yanomami (54% >10 mm) confirmed a significantly higher frequency of anergy among the Yanomami patients (Fig. 2). The age distribution of PPD skin reactions showed an increased percentage of reactions <5 mm among young children and elders, independent of BCG status.

Antibodies to *M. tuberculosis* Antigens. All Yanomami sera collected were analyzed for specific antibodies reactive to two glycolipids unique to *M. tuberculosis* cell wall, DAT and PGL-Tb1 (8, 9). Sera from 71% of individuals had IgM antibodies reactive to DAT and 59% to PGL-Tb1 antigens. These values are significantly higher ($P < 0.001$) than those found among populations of European descent from the same region (Fig. 3). Similarly, among the tuberculosis patient populations, a significantly higher percent of Yanomami had IgM antibodies (58% to DAT and 54% to PGL-Tb1) in comparison with patients of European descent (6% to DAT, and 4% to PGL-Tb1). In contrast, whereas 47% and 27% of the control patient populations produced IgG antibodies to these glycolipids, none in the Yanomami patient population developed IgG antibodies to PGL-Tb1 and only 38% to DAT (Fig. 3). Of particular interest was the IgG4 isotype that has been reported to be associated with interleukin-4-dependent responses (16). Of the serum samples from tuberculosis patients, titers of IgG4 antibodies reactive to DAT were significantly higher among the Yanomami than among the control population of other Brazilians ($P < 0.0001$) (Fig. 4). Thus, in contrast to their diminished cell-mediated immune responses to tuberculin, the Yanomami Indians had higher levels of IgM antibodies and TH2-dependent IgG antibodies to *M. tuberculosis* glycolipid antigens. Within the Yanomami population with tuberculosis, a negative correlation was observed between PPD responses and antibodies of the IgM isotype ($r = 0.471$, $P = 0.018$) and IgG ($r = 0.395$, $P = 0.051$).

Antibodies to Other Infectious Agents. Serum samples randomly chosen from the total population were tested against several nonmycobacterial antigens to estimate contact with outside populations and exposure to other pathogens. Almost all individuals tested (198 of 202) had antibodies to herpes simplex virus type I, but only 3% had antibodies reactive to herpes simplex virus type II. An estimated 39% of the population had IgG antibodies reactive to *Toxoplasma gondii*, whereas none was positive for antibodies to *Treponema pallidum*. Samples chosen among the PPD anergic population, with active tuberculosis infection and/or recently BCG vaccinated, were tested for antibodies to infectious agents associated with immunodeficiency diseases that could be responsible for the PPD anergy. No antibodies against HIV gp160 or P24 were detected, nor were antibodies against measles virus observed.

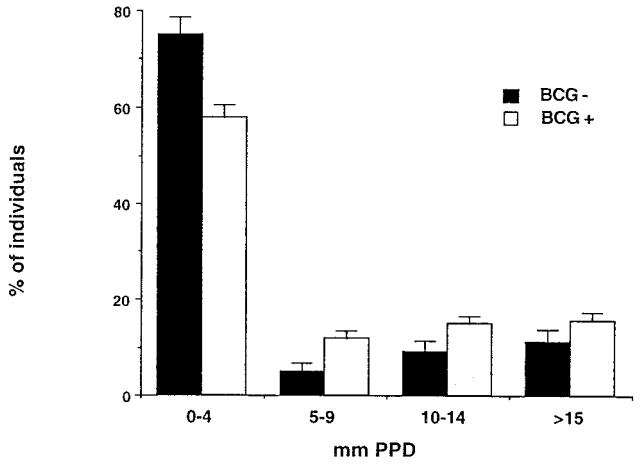

FIG. 1. Distribution of tuberculin PPD skin reactions among the Yanomami population. BCG-vaccinated ($n = 411$) (empty bars); non-BCG-vaccinated ($n = 144$) (filled bars).

DISCUSSION

Evidence of tuberculosis in humans has been dated as early as 6,000 BC (16), and restriction fragment length polymorphism evidence indicates the existence of the *M. tuberculosis* complex in the Western Hemisphere earlier than the 14th century (17). With a case fatality rate for untreated disease of 50% in virtually every population studied, one would predict that first contact with tuberculosis of a naive population would lead to a raging epidemic with devastating consequences. Yet, very few epidemics of tuberculosis in naive populations have been amenable to study. The best characterized are those among the Eskimos (18) and among the Northwest American Indians (19), both of which were studied only during the later phases of the epidemic. The early dynamics and immunologic responses of naive populations to tuberculosis are largely unknown. The present assessment of the tuberculosis spread among the Yanomami in their accelerating contact with Brazilian miners offered a unique opportunity to study the development of an epidemic in an isolated population with their first contact with tuberculosis. Although access to modern laboratory facilities was not available and a number of sophisticated immunological tests could not be carried out, clinical studies of the epidemiology, mycobacteriology, and immunological responses within one sector of the Yanomami reservation, has allowed a glimpse into the early phases of a tuberculosis epidemic and some intriguing inferences about the nature of the interaction of *M. tuberculosis* with a previously naive population. The first conclusion that can be drawn

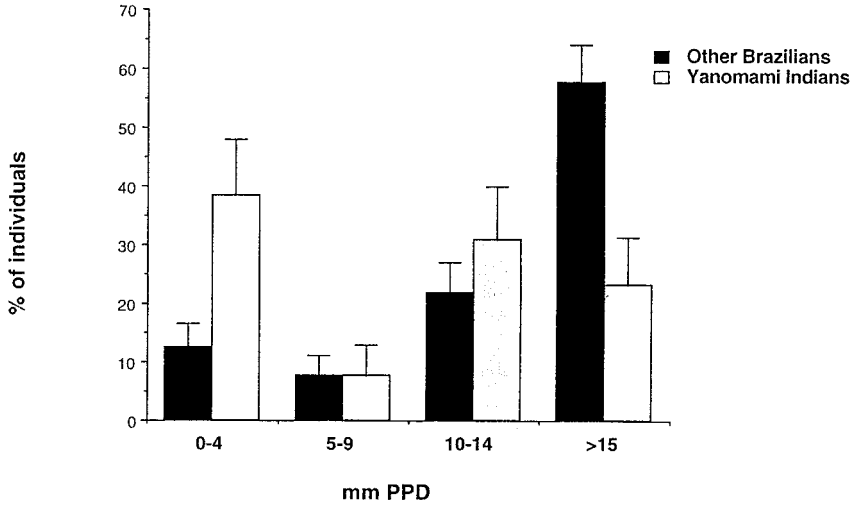

FIG. 2. Distribution of PPD reactions among active pulmonary tuberculosis patients. Yanomami Indian population (n = 26) (empty bars); other Brazilian patients of European descent (n = 42) (filled bars).

from this study is that tuberculosis is present in epidemic proportions among the Yanomami population of Brazil, estimated to have a high mortality rate reflecting the severity of the disease. The prevalence rate of 6.4% is 100-fold higher than that observed in the Amazonian State in general (68 per 100,000) (20). Incidence rates appear to be similarly extraordinarily high. The finding of few restriction fragment length restriction patterns and a phage lysotype profile similar to that of strains found locally suggest that the current Yanomami epidemic has been introduced by recent contact with local people. Our findings suggest that the Yanomami are experiencing the beginning of an epidemic, which from contemporary modeling studies, in the absence of appropriate medical intervention, would be predicted to last for at least a century (21, 22) and be devastating to this indigenous population.

It is notable that control individuals of European extraction living within the same general region had a higher degree of tuberculin reactivity. This finding supports the view that the defective cell-mediated immune responses and high rates of antibodies seen in the Yanomami population are likely to be host-dependent phenomena and not a peculiarity of the predominant *M. tuberculosis* strain in the region.

A second major finding is that, in contrast to the 90% of positive tuberculin reactions found during the epidemics in this century among the Alaskan and Canadian Eskimo and northwest Indian populations, the proportion of positive tuberculin responders among the Yanomami Indians was surprisingly low (27%). The pattern of unresponsiveness was reflected in the significantly higher frequency of skin test negative patients among Yanomami tuberculosis cases (46%) than within the control group (20%). Similarly, the frequency of anergy to PPD was 58% among recently BCG-vaccinated individuals, an extraordinarily high frequency of unresponsiveness. Studies of otherwise healthy populations have shown rates of unresponsiveness to PPD of active tuberculosis patients varying from 9.5% (23, 24) to 25% (25). By using the same source of tuberculin PPD we found that 72% of army recruits, of an average age of 18 years, who had been BCG-vaccinated at birth, were still PPD positive. Thus the failure of the Yanomami to respond cannot be attributed to the nature of the PPD or the BCG vaccine used throughout the country. There was no evidence for HIV infection, measles infection, mononucleosis, malnutrition, or alcoholism in the Yanomami population, thus excluding common conditions known to lead to immunocompromise. Thus we are unable to adduce any other reason for the skin test unresponsiveness after tuberculosis infection or BCG immunization than an intrinsic unresponsiveness of this population on first exposure to mycobacterial antigens.

In contrast to their diminished cell-mediated immune responses, the Yanomami population in general, and the patients with tuberculosis in particular, had surprisingly high levels of antibodies to *M. tuberculosis* glycolipid antigens. The vast majority of these antibodies were found to be of the IgM isotype, generally a T-cell independent class of antibodies. Of the IgG antibodies produced to the DAT glycolipid antigen, levels of IgG4 isotypes were significantly elevated, which is

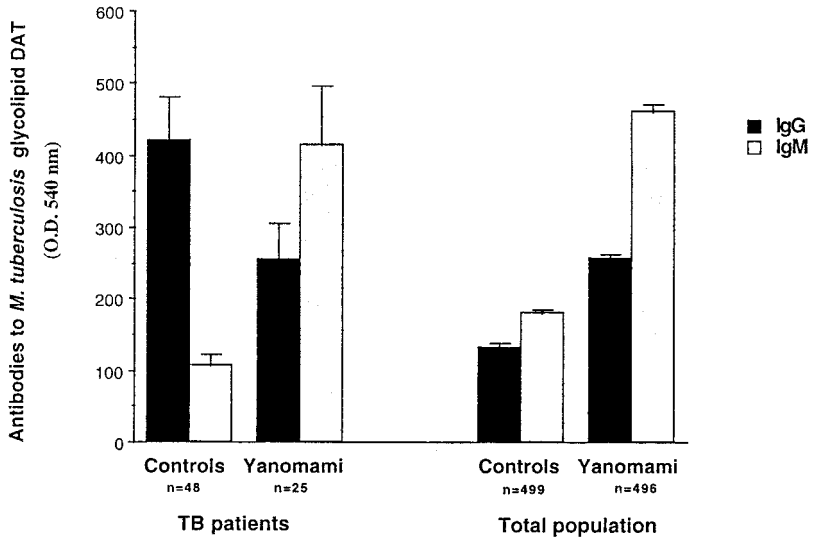

FIG. 3. *M. tuberculosis* glycolipid DAT antibodies in tuberculosis patients and total population among Yanomami Indians and a control population of other Brazilians. Total antibodies is measured by OD 540 nm of protein. The Yanomami have lower IgG DAT antibodies (P = .038) among the tuberculosis patients (*Left*), but higher DAT IgG in the general population (*Right*) ($P < 0.001$). In contrast, the IgM DAT antibodies in the Yanomami are elevated ($P < 0.001$) in both tuberculosis patients (*Left*) and in the total population (*Right*).

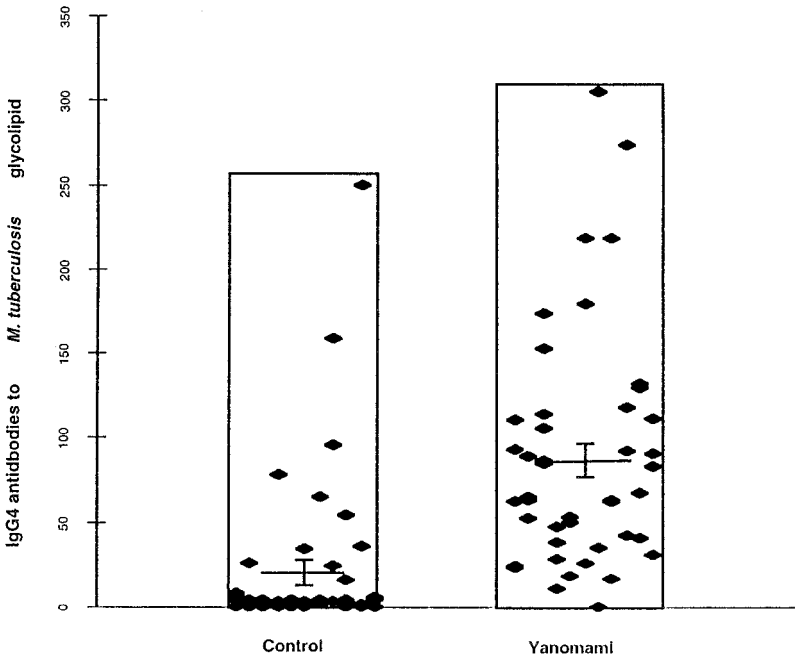

FIG. 4. Levels of IgG4 antibodies to *M. tuberculosis* glycolipid DAT. The Yanomami Indians (*Right*) have significantly higher IgG4 antibodies than the control Brazilian population (*Left*) ($P < 0.001$).

reported to be interleukin-4-dependent (15). Without detailed laboratory studies on T-cell subsets and patterns of lymphokine production, which was not possible because of the remoteness of the Yanomami reservation and the nature of the medical mission, the precise reasons for the failure to develop appropriate cell-mediated immune responses cannot be established with certainty. Nevertheless, it is reasonable to infer that the response of this population to its first exposure to *M. tuberculosis* or BCG infection has been to produce a lower level of cell-mediated immunity and a higher level of antibodies than has been found in other populations that have had a longer history of exposure to tuberculosis. Within the framework of contemporary immunology, that pattern of host response suggested the existence of a broad spectrum of immune reactions, with a greater tendency within some individuals from this population toward TH2 responses that are not likely to be protective against tuberculosis.

There is precedent for such a dichotomy of human immune responses to mycobacteria in the spectrum of leprosy (26–28). At one pole of the spectrum, tuberculoid leprosy, patients mount a strong TH1 type cell-mediated immune response, produce interferon-γ and interleukin-2 in lesions and localize the infection, albeit with the consequence of damage to nerves. At the opposite pole, in lepromatous leprosy, there is diminished cell-mediated immunity and high titers of antibodies, which result in failure to restrict the growth of the pathogen and deposition of immune complexes leading to erythema nodosum leprosum. The striking correlation between the ability to generate cell-mediated immune responses and to restrict the growth of *M. leprae* (29) has not been established, however, in human tuberculosis (30–33). For example, BCG immunization produces tuberculin skin test reactivity in most populations, but its protective efficacy against pulmonary tuberculosis has varied widely in different parts of the world (33). Similarly, it has been difficult to establish a correlation between tuberculin reaction size and degree of protection. Nevertheless, in untreated tuberculosis, a significant proportion of patients are found to be anergic or immunologically unresponsive to tuberculin, and that group traditionally has been associated with more severe disease (34).

It is our hypothesis that in populations with long exposure to tuberculosis before the availability of antituberculous treatment in the 1950s, *M. tuberculosis* infection exerted a powerful genetic selective pressure, resulting in the elimination of a significant proportion of highly susceptible individuals during their reproductive age. The weak cell-mediated immunity to tuberculosis found among the Yanomami suggests that this population was immunologically naive before exposure. The present data suggest that the first exposure to infection by the tubercle bacillus in a naive population, similar to leprosy, engenders a spectrum of host immunologic responses. Individuals producing poorly protective responses subsequently would be selected against. Those individuals producing a higher degree of cell-mediated immunity, including TH1 responders, would be selected for and ultimately would become the predominant phenotype of the population.

Questions as to what extent the low level of cell-mediated immunity and high frequency of antibodies is an intrinsic genetic characteristic of the Yanomami people, the result of concurrent infections such as by helminths that stimulate predominantly TH2 responses, or represents a lack of negative selection by pathogens requiring cell-mediated immunity for survival, remain intriguing ones. Further insight into the origins of the unique immune spectrum of the Yanomami Indians tuberculosis infection will require detailed analysis of cellular immune mechanisms and genetic polymorphisms of this extraordinary population. Nevertheless, the present results do suggest that *M. tuberculosis* infection may have exerted a powerful selective pressure, shaping the human immune responses to infection and perhaps the human genome.

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