

Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity *in vivo*

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Activation of Th1 lymphocytes, IFN- γ production and macrophage activation are crucial in defense against *Mycobacteria*. In developing countries, Th2 activation and IL-4 production have been associated *in vitro* with tuberculosis and with poor clinical outcome after treatment. Serological markers of Th1 [soluble lymphocyte activation gene (LAG)-3] and Th2 (IgE, soluble CD30, and CCL22/macrophage-derived chemokine) activity were measured in 414 HIV-negative tuberculosis patients from The Gambia and Guinée and in 414 healthy household and community controls. Measurements were repeated during treatment to assess the effect of therapy on Th1/Th2 ratio. At diagnosis, sLAG-3 levels were lower in patients than in community controls ($p < 0.0001$), but were higher in household controls exposed to contact with patients than in community controls ($p < 0.0001$). In comparison with community controls, patients had consistently higher levels of IgE, sCD30, and CCL22 ($p < 0.0001$), whereas household controls had lower levels of indicators of Th2 activity ($p < 0.0001$). After treatment, cured patients had higher levels of Th1 ($p < 0.0001$) and lower levels of Th2 ($p < 0.0001$) activity than patients who were not successfully treated or interrupted therapy. In Africa, tuberculosis is associated with low Th1 and high Th2 activity *in vivo*, whereas close exposure to tuberculosis is associated with a high Th1/Th2 ratio. Patients with favorable outcome after treatment exhibit a higher Th1/Th2 ratio compared to patients with poor clinical outcome.

Key words: Tuberculosis / Th1/Th2 activity / Lymphocyte activation gene-3 (LAG-3) / IgE / Soluble CD30

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1 Introduction

Tuberculosis is a major health problem worldwide with an estimated 7.5 million new cases each year, more than 95% of these in developing countries [1]. The outcome of mycobacterial infection is a dynamic process that depends on factors associated with the pathogen, the

host and the environment. The first line of host defense against *Mycobacteria* is provided by macrophages through nonspecific mechanisms of resistance, but 2–3 weeks after infection, T cell-mediated protective immunity develops. Although all three T cell subsets, CD4 $\alpha\beta$ ⁺, CD8 $\alpha\beta$ ⁺ and $\gamma\delta$ ⁺ cells may participate in protection against *Mycobacteria* [2], activation of macrophages, which is crucial for elimination of *Mycobacteria*, depends on the T helper type-1 (Th1) cytokines IFN- γ and TNF- α [3, 4]. Studies of patients with familial susceptibility to mycobacterial infections have confirmed that Th1 responses are essential for protection [5]. Mutations affecting the expression of IFN- γ receptors, IL-12 or IL-12 receptors have been associated with disseminated infections with environmental *Mycobacteria* or BCG. In most cases, severe and often fatal disease is associated

[1 22823]

The first two authors equally contributed to this study.

Abbreviations: sCD30: Soluble CD30 sLAG-3: Soluble lymphocyte activation gene-3 MDC-CCL22: Macrophage-derived chemokine GM: Geometric mean CI: Confidence interval

with the absence of production of, or response to, IFN- γ . In case of a partial deficit, disease is less severe [5–7]. Those observations suggest a correlation between the magnitude of Th1 responsiveness and control of mycobacterial infection, however, this has not been studied *in vivo* until now.

The patterns of released cytokines during specific immune responses are characteristic for distinct subsets of CD4⁺ T helper cells, whose polarized forms are Th1 and Th2 [8, 9]. Th1 cells produce IFN- γ , but not IL-4, express cytotoxicity, promote cell-mediated immunity, macrophage activation and phagocytosis. Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, but not IFN- γ , induce B cell differentiation with production of all Ig isotypes (including IgE), and inhibit several macrophage functions. However, most of CD4⁺ Th cells express a mixed pattern, designated as Th0, that exert intermediate effects depending on the ratio of cytokines produced and the nature of responding cells. Th cell subsets express different surface molecules and chemokine receptors. Activated Th1 cells express on the membrane and release in soluble form the lymphocyte activation gene (LAG)-3 molecule [10], whereas activation of Th2 results in membrane expression and release of CD30 both *in vitro* and *in vivo* [11, 12]. Since lymphocyte stimulation with IL-12 resulted in preferential activation of CD30⁺ T cells, it has been suggested that CD30 might associate with IFN- γ production [13]. However, subsequent studies in mice confirmed a strong association between CD30 expression and Th2 cytokine profile [14]. It has recently been shown that macrophage-derived chemokine (MDC/CCL22), a chemoattractant for monocytes, dendritic cells, and activated Th2 lymphocytes, is secreted preferentially by Th2-like cells, and high CCL22 plasma levels were found in Th2-dominated conditions [15, 16]. *In vivo* measurements of IFN- γ and IL-4 would theoretically be the best way to assess whether a Th1/Th2 imbalance represents a host factor for developing tuberculosis or poor clinical outcome of this disease. These cytokines, however, are short-range molecules that are rapidly bound by their receptors and/or inactivated by proteases, and their plasma levels are difficult to interpret. Therefore, assays have been developed to measure indirect indicators of preferential Th1 or Th2 activity *in vivo*.

The aim of this study was to investigate the Th1/Th2 ratio *in vivo* of tuberculosis patients at time of diagnosis and after completion of treatment. For this purpose, we measured markers of Th1 and Th2 activity in HIV-negative tuberculosis patients in West Africa and compared with the levels of these markers obtained in randomly selected household and community healthy controls. Plasma levels of IgE, soluble CD30 (sCD30) and CCL22

were used as indicators of Th2 activity, and levels of soluble LAG-3 (sLAG-3) as a parameter of ongoing Th1 activity.

2 Results

A total of 414 patients with pulmonary tuberculosis were recruited at diagnosis, together with their paired household and community controls (Table 1A). In patients, the mean plasma level of sLAG-3 was significantly lower than that measured in healthy community controls [geometric mean, (GM) ratio: 0.46, 95% confidence interval (CI): 0.41–0.53], whereas in healthy household controls, the mean sLAG-3 level was significantly higher than that found in community controls (GM ratio: 2.84, 95% CI: 2.49–3.23) (Fig. 1). For Th2 markers, the mean plasma levels of IgE, sCD30, and CCL22 in tuberculosis patients were consistently higher than those found in community controls (GM ratios: 1.60, 95% CI: 1.37–1.87 for IgE; 1.65, 95% CI: 1.54–1.78 for sCD30 and 2.17, 95% CI: 2.01–2.32 for MDC/CCL22). Household controls had lower levels of Th2 indicators than community controls (GM ratios: 0.45, 95% CI: 0.39–0.53 for IgE; 0.69, 95%

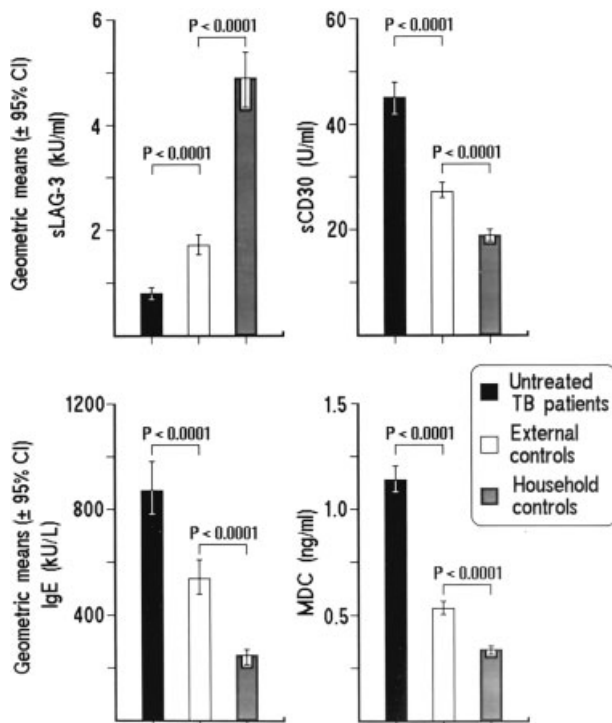


Fig. 1. Plasma levels of sLAG-3, IgE, sCD30, and MDC/CCL22 measured in 414 patients with untreated pulmonary tuberculosis and in their paired 414 community (X) controls and 414 household (H) healthy controls. Results represent the geometric means ($\pm$ 95% confidence intervals).

Table 1. Demographic and clinical data of matched patients, household and community controls included in the study (*n* = 414)

		Tuberculosis patients	Household (H) healthy controls	Community (X) healthy controls
A. Demographic data				
Country	The Gambia	285 (69) ^{a)}	285 (69)	285 (69)
	Guinée	129 (31)	129 (31)	129 (31)
Ethnic group	Mandinka	112 (27)	110 (27)	112 (27)
	Fula	93 (22)	91 (22)	94 (23)
	Jola	51 (12)	55 (13)	60 (14)
	Sousou	47 (11)	47 (11)	48 (12)
	Wolof	37 (9)	38 (9)	21 (5)
	Other	74 (18)	73 (18)	79 (19)
Age	15–24	160 (39)	168 (41)	126 (30)
	25–39	178 (43)	178 (43)	204 (49)
	> 40	76 (18)	68 (16)	84 (20)
Sex	Male	299 (72)	200 (48)	219 (53)
	Female	115 (28)	214 (52)	195 (47)
B. Clinical data				
BCG scar	Present	249 (60)	239 (58)	243 (59)
	Absent	165 (40)	175 (42)	171 (41)
History of tuberculosis	Yes	18 (4)	8 (2)	3 (1)
	No	396 (96)	406 (98)	411 (99)
Self-reported wheeze	Yes	38 (9)	13 (3)	22 (5)
	No	376 (91)	401 (97)	392 (96)
History of asthma	Yes	8 (2)	4 (1)	7 (2)
	No	406 (98)	410 (99)	407 (98)
History of worms	Yes	121 (29)	131 (32)	134 (32)
	No	293 (71)	283 (68)	280 (68)
Helminth infestation ^{b)}	Present	45 (48)	11 (27)	8 (26)
	Absent	49 (52)	29 (73)	24 (75)
	Not tested	35	89	97
PPD skin test response	< 10 mm	45 (11)	153 (38)	255 (53)
	> = 10 mm	353 (89)	254 (62)	149 (37)
	Not tested	16	7	10

a) Percent values in parentheses.

b) Assessed in Guinée only.

Table 2. Interaction of helminth infestation with case/control status for sCD30 (data from Guinée)^{a)}

Individuals tested		Geometric means (GM) for sCD30 (U/ml)	Ratio of geometric means (95 % CI) for helminth infection stratified by case control status
Community control:	Negative	24.4	1
	Positive	41.6	1.68 (1.16–2.43)
Household control:	Negative	23.1	1
	Positive	35.0	1.52 (1.10–2.11)
Tuberculosis patients:	Negative	52.3	1
	Positive	47.3	0.92 (0.76–1.12)

a) p -value for interaction $\chi^2 = 11.68$ (df=2), $p=0.003$. Ratio of geometric means for household versus community control and index case versus community control amongst those negative for helminth infection are 0.94 (0.74–1.19) and 2.16 (1.73–2.68), respectively.

CI: 0.64–0.75 for sCD30 and 0.63, 95%CI: 0.59–0.67 for CCL22, respectively). The same pattern of a low Th1/Th2 ratio in patients and a high Th1/Th2 ratio in household controls when compared to community controls was observed in each country when data were analyzed separately.

In order to adjust for potential confounding, we conducted multivariate analysis including the following variables: age (to assess for residual confounding), sex, ethnic group, presence of BCG scar, former tuberculosis, history of wheezing, past and current infestation with helminths, smoking, PPD skin test positivity (Table 1B). These variables had no effect on the relationship between case/control status and Th1/Th2 parameters (data not shown). There was, however, an interaction between case/control status and infestation with hel-

minths for the sCD30 marker ($p=0.003$). In both household and community controls, sCD30 levels were higher in the presence of concurrent helminth infection than in its absence (GM: 35.0 vs. 23.1 U/ml and 41.6 vs. 24.4 U/ml, respectively), whereas sCD30 levels were similar in tuberculosis patients, whether coinfecting with helminths (47.3 U/ml) or not (52.3 U/ml) (Table 2).

Tuberculosis patients were followed-up during and after treatment. Out of 414 patients, 209 were re-tested at 2–3 months and 240 at 6–8 months, in whom outcome of treatment was documented. As shown in Table 3, the levels of sLAG-3 measured after 2–3 months of treatment were nearly four times higher than before treatment and nearly six times higher at the end of therapy ($p<0.0001$), up to a level higher than that found in community controls at baseline (GM: 1.72 kU/ml). In parallel

Table 3. Geometric means of Th1/Th2 parameters at baseline, 2/3 months and 6/8 months follow-up

Parameter		Geometric mean at 2/3 months follow-up ($n = 209$)	Geometric mean at 6/8 months follow-up ($n = 240$)
sLAG-3 (kU/ml)	At baseline	0.622	0.739
	At follow-up	2.549	4.100
IgE (kU/l)	At baseline	862.8	852.6
	At follow-up	496.1	288.3
sCD30 (kU/ml)	At baseline	48.5	46.2
	At follow-up	31.9	18.0
CDL22/MDC (ng/ml)	At baseline	1.21	1.21
	At follow-up	0.78	0.47

with increased Th1 activity, a remarkable decrease of Th2 parameters was found as treatment progressed. Levels of Th2 markers were concordantly reduced by about one third after 2–3 months and by almost two thirds at the end of treatment ($p < 0.0001$), with values that were lower than those found in community controls at baseline (GM IgE: 543.4 kU/l, sCD30: 27.1 U/ml, CCL22: 0.534 ng/ml) and quite similar to those measured in household controls (GM IgE: 244.6 kU/l, sCD30: 18.7 U/ml and CCL22: 0.334 ng/ml, respectively).

To assess whether fluctuations of Th1/Th2 parameters in patients occurred randomly and not in relation to treatment, parameters were re-tested in 37 household and 34 community controls 6 months after their baseline samples had been collected. For community controls, there were no difference between 6 months and baseline values for any of the three Th2 parameters (GM IgE: 545 vs. 547 kU/l; sCD30: 25.4 vs. 23.5 U/ml; CCL22: 0.537 vs. 0.532 ng/ml), whereas sLAG-3 at 6 months was higher than at baseline (GM 3.13 vs. 2.16 kU/ml, $p < 0.05$). In household controls, Th2 indicators at 6 months were significantly higher than those measured at baseline (GM IgE: 380 vs. 175 kU/l, $p = 0.0007$; sCD30: 23.3 vs. 17.2 U/ml, $p = 0.002$; CCL22: 0.522 vs. 0.274 ng/ml, $p < 0.0001$), whereas sLAG-3 was significantly lower than its baseline value (GM 4.33 kU/ml vs. 5.93 kU/ml, $p = 0.0003$). This suggests that in household controls, Th1/Th2 ratio tended to become similar to that of community controls, presumably because their immediate exposure to *M. tuberculosis* was removed during the treatment of their diseased contact.

To estimate further the effect of treatment, geometric mean plasma levels of Th1 and Th2 markers were compared between “cured” and “non-cured” patients, using the dichotomized outcome of treatment variable (Fig. 2). There was a remarkable difference in the levels of Th1 and Th2 markers between cured and non-cured patients at the end of treatment, adjusted for their baseline level. Cured patients showed significantly higher levels of sLAG-3 than those non-cured (GM ratio; 2.17 95% CI 1.67–2.81, $p < 0.0001$), reflecting a higher Th1 activity after effective tuberculosis treatment. In cured patients, the dramatic increase of sLAG-3 levels was paralleled by a substantial decrease of all the Th2 parameters ($p < 0.0001$) at the end of treatment (GM ratios: 0.44, 95% CI: 0.31–0.62 for IgE; 0.51, 95% CI: 0.41–0.68 for sCD30 and 0.44, 95% CI: 0.37–0.55 for CCL22). In non-cured tuberculosis patients, however, Th2 markers remained at high levels during treatment, probably reflecting the persistence of active *M. tuberculosis* infection in these individuals.

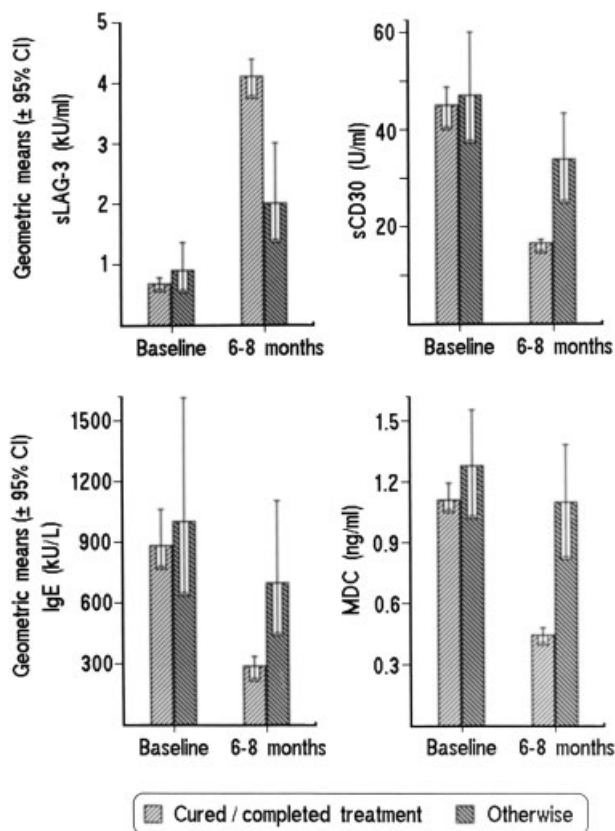


Fig. 2. Plasma levels of sLAG-3, IgE, sCD30, and MDC/CCL22, measured in tuberculosis cases before and after antimycobacterial treatment (6–8 months). The outcome of treatment is dichotomized as “cured” (cured and/or completed treatment) versus “otherwise” to indicate non-cured patients due to treatment failure and/or interruption of treatment before completion. Results represent the geometric means ($\pm 95\%$ confidence intervals).

Since clinical outcome could be appropriately documented in only 60% of tuberculosis patients, we investigated whether potential bias might have resulted from losses to follow-up. Age, sex, ethnic group and baseline values of Th1/Th2 parameters were compared for patients who had been completely followed-up and those who had incomplete clinical documentation. There was no difference in age, ethnic group or baseline values of Th1/Th2 parameters between those who completed follow-up and those who did not. Although males were less likely to be appropriately followed-up than females (55% vs. 66%, $p = 0.04$), there was no evidence that outcome of treatment differed between males and females ($p = 0.65$).

3 Discussion

The low plasma levels of sLAG-3 and the high levels of IgE, sCD30 and CCL22 found *in vivo* in almost all patients with untreated pulmonary tuberculosis suggest that tuberculosis in Africa is associated with a reduced Th1 activity and a concomitantly increased Th2 activity. Conversely, the opposite pattern observed in household controls, who were in contact with the tuberculosis cases, suggests that a high ratio between Th1 and Th2 markers *in vivo* is associated with recent exposure to *M. tuberculosis*. The levels of these markers in community controls should reflect the background level of Th1/Th2 activity in the general population in Africa. Since membrane expression and release of sLAG-3 by activated IFN- γ -producing Th1 (and Th0) cells is an IFN- γ -dependent phenomenon [10], these data represent *in vivo* correlates of results from *in vitro* studies showing that patients with active tuberculosis have poor production of IFN- γ [3, 17, 18] and that recent exposure to infectious tuberculosis is associated with increase of Th1 activity [19]. Whether decreased production of IFN- γ *in vitro* in untreated tuberculosis is associated with a concomitant increase of the production of Th2 cytokines is controversial [3, 18, 20–22]. A clonal study of PPD-specific T cell response in active tuberculosis showed that the T cell response to mycobacterial antigens included the activation of IL-4-producing T cells and the generation of PPD-specific Th2 clones [23]. It has recently been shown that increased IL-4 mRNA correlates with increased IgE and sCD30 in tuberculosis patients [24]. The high levels of IgE, sCD30 and CCL22 (all IL-4-dependent molecules) observed in tuberculosis patients provide *in vivo* evidence for Th2 activation, as suggested by *in vitro* studies in patients from developing countries, in whom IL-4 expression could be easily detected [20, 22].

As in all case-control studies, however, the data presented here do not provide evidence of a causal effect. It is difficult to say whether it is primarily low IFN- γ production which allows high Th2 activity and disease onset or if it is expanded Th2 function induced by host factors, which is primarily responsible for down regulation of Th1 activity and clinical disease in *M. tuberculosis*-infected individuals. Clinical conditions such as atopy or helminth infection are known to induce Th2-type immune responses [12]. In this study, no difference was found in the proportion of individuals among cases and controls reporting history of wheeze, asthma, or worm infection. Current helminth infection was however, more frequent in tuberculosis patients (17%) than in household (6%) or community (9%) controls (Table 1B). Therefore, we evaluated the potential impact (confounding or effect modification) of conditions inducing Th2-type immune

response on the levels of Th1/Th2 markers according to case-control status. No evidence of interaction was found between history of any of the three conditions and case-control status for the Th1 or Th2 markers tested, that is, IgE or CCL22 levels were similarly increased and sLAG-3 levels decreased in both cases and controls reporting history of worm infestation, reflecting the background effect of helminth infection on individual immune responses (data not shown). An interaction was observed, however, between case/control status and current worm infestation for the sCD30 marker. The higher sCD30 values observed in helminth-infected controls probably reflects the Th2-inducing effect of intestinal helminths, but this effect was probably masked in tuberculosis patients by Th2 activity which was already high, possibly due to mycobacterial infection.

Overall, we observe a marked contrast between low Th1 and high Th2 response in tuberculosis patients as compared to community controls and a high Th1 and low Th2 response in household controls exposed to *M. tuberculosis*. Our data are not in conflict with the hypothesis that the high prevalence of helminthiasis in developing countries impairs protective immunity against *Mycobacteria* [25]. The sCD30 results we observed, however, suggest that intestinal helminths increase the Th2 bias of tuberculosis patients, but this would need further investigation. The hypothesis that patients with tuberculosis develop a low Th1/Th2 ratio during the initial phases of the disease could be related to mechanisms inducing either the preferential expansion of Th2-like cells or the decrease of the Th1 activity or both. Recent *in vitro* data indicate that apoptosis of CD4 T cells could play a role in the defective IFN- γ production observed both in tuberculosis patients [26] and in experimental tuberculosis [27]. Since Th1 clones are more susceptible to apoptosis than Th2 clones [28], the Th2 dominance observed in active tuberculosis may be related to death of a number of Th1 cells. Alternatively, the decreased Th1 function may be related to suppressive cytokines. Transforming growth factor- β and IL-10 are produced during active tuberculosis and may play an important role in down regulation of IFN- γ at disease onset [3, 18, 29, 30].

Clinical healing induced by anti-mycobacterial therapy was associated with a shift towards high Th1 activity, resulting in a pattern resembling that found in exposed individuals who remained healthy. Follow-up during treatment showed that the increase of sLAG-3 levels and the decrease of IgE, sCD30 and CCL22 levels during therapy were only partial after 2–3 months, and that full treatment was required for tuberculosis patients to achieve a Th1/Th2 ratio comparable to that of healthy household controls, despite the fact that different schedules and dosages were used in each country during the

second phase of treatment. These *in vivo* data are fully in agreement with the *in vitro* observation that poor IFN- γ production in untreated patients is augmented by anti-mycobacterial therapy [17–19] and that Th1 polarization of the T cell responsiveness to either PPD or other antigens occurs in tuberculosis patients with favorable clinical outcome [23]. They also confirm in a large number of patients and controls the observation that African tuberculosis patients have elevated IgE levels that decrease with therapy [31]. Interestingly, patients who suffered poor clinical outcome and/or failed to comply with their treatment schedule had only small rises in sLAG-3 concentrations during treatment, whereas the indicators of Th2 activity remained high, or decreased only moderately, in comparison with the values observed before treatment. This is consistent with the *in vitro* observation that the Th2/Th0 shift to Th1 of both the PPD-specific and the bystander T cell response failed to occur in patients who had treatment failure [23]. Since lack of Th1 polarization is associated with treatment failure, we hypothesize that a Th0/Th2 response in tuberculosis, even if not detrimental, represents an inefficient mechanism to induce pathogen clearance and healing. Our data, showing a high Th1/Th2 ratio in both exposed contacts who remained healthy and in tuberculosis patients with favorable outcome, encourage further investigation of possible ways to achieve early Th1 polarization of T cell responses in tuberculosis patients in order to accelerate healing.

4 Materials and methods

4.1 Patients and controls

The study was carried out simultaneously in two countries in West Africa (The Gambia and the Republic of Guinée), after approval by the appropriate Ethical Committees. Details of the methods are given elsewhere [32]. Newly diagnosed smear positive pulmonary tuberculosis cases older than 15 years were eligible for the study and were recruited after giving informed consent. Pulmonary tuberculosis was defined by the presence of acid-fast bacilli in two consecutive sputum samples, or one positive sputum confirmed by positive culture for *M. tuberculosis*. A blood sample was obtained from each case for immunological analysis within 2 weeks of diagnosis. For each patient enrolled, two control subjects were recruited: first, a healthy control age-matched within 10 years was selected at random within the household of the patient, and a second control, similarly matched, was recruited at random in the community from a nearby household. Chest X-rays and tuberculin skin tests were performed at enrolment. Patients and controls gave informed consent for HIV testing and individuals positive for HIV1 or HIV2 were appropriately counseled and excluded from the study. Stool samples were obtained from patients and con-

trols in Guinée, for the detection of intestinal parasites. To decrease the risk of under-diagnosis related to the collection of a single stool sample, patients and controls were interviewed on actual presence of worms and recent past history of specific anti-worm treatment. All tests were performed blind to the case-control status of the subjects.

Directly observed therapy of tuberculosis patients, including rifampin, isoniazid, pyrazinamide and ethambutol, was given during the first 2 months, followed by rifampin and isoniazid three times weekly for an additional 4 months in The Gambia or daily for another 6 months in Guinée. At the end of treatment, clinical healing was assessed by clinical examination (disappearance of symptoms, weight gain) and conversion of sputum smears to negative. In case of treatment failure, patients were re-treated in each country according to the national tuberculosis control program recommendations. Patients were followed-up during treatment and venous blood samples were obtained after 2/3 months and at the end of treatment. A blood sample was also collected from a sub-sample of household and community controls 6 months after their enrolment in the study for comparison with baseline Th1/Th2 ratio.

4.2 Measurement of soluble LAG-3, soluble CD30, IgE and MDC/CCL22

Plasma sLAG-3 levels were assessed by an ELISA method [10, 32]. sLAG-3 levels were indicated in arbitrary units using a pool of culture supernatants from activated Th1 T cell clones containing high concentrations of sLAG-3 (100 arbitrary kU/ml) as a reference reagent [33]. Commercial assays were used to measure sCD30 (Dako, Glostrup, Denmark), total IgE (CAP System, Pharmacia, Uppsala, Sweden), and MDC/CCL22 levels (R&D Systems, Minneapolis, MN). For each parameter, values represent the mean of duplicate determinations in each sample.

4.3 Statistical analysis

The distribution of all parameters was normalized by transforming to their natural logarithms. Results are reported as geometric means and the ratio of geometric means (GM ratio), with 95% confidence intervals (CI). The analysis of Th1 and Th2 parameters in cases and controls at recruitment used random effects linear regression to control for the matching in the design and to adjust for the effects of potential confounding variables. Th1 and Th2 parameters in tuberculosis patients during and at the end of treatment were compared separately with their baseline values using a paired *t*-test. The effect of treatment on Th1/Th2 markers at the end of follow-up was assessed using linear regression, adjusted for baseline values. It is usual to equate completion of tuberculosis treatment with cure, as many patients without symptoms after treatment cannot produce sputum to test. Accordingly, we dichotomized outcome of treatment as

“cured” (cured or completed treatment) versus “non-cured” (treatment failure or interruption of treatment before completion).

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