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ORIGINAL ARTICLE

Tuberculous Lymphadenitis: Skin Delayed-Type Hypersensitivity Reaction and Cellular Immune Responses

Lymphadénite Tuberculeuse: Peau Réaction d'hypersensibilité Retardée de Type et les Réponses Immunitaires Cellulaires

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ABSTRACT

BACKGROUND: **Tuberculous lymphadenitis (TL) is the commonest form of extra-pulmonary tuberculosis in tropical countries.**

OBJECTIVE: **This study aimed to characterize *in vivo* and *in vitro* cellular immune responses to *Mycobacterium* PPD in TL patients as markers of disease and healing.**

METHODS: **Following informed consent, 36 TL patients, 40 patients with pulmonary tuberculosis (TB) and 20 apparently healthy individuals were enrolled when they met specific selection criteria. The tuberculin skin test (TST) and peripheral blood mono-nuclear cells (PBMCs) culture were conducted using PPD. The cytokines were measured using commercial kits.**

RESULTS: **The mean TST was 24.6 ±8.0 mm for TL patients. The TST was variable in pulmonary TB patients and healthy individuals. It was reactive in a third of pulmonary TB patients with a mean of 20 ±3.0 mm and reactive in half of the healthy individuals with a mean of 12.6 ±3.2 mm. Pre and post-treatment interferon gamma (IFN-γ) mean levels were 498.6 ±905.8 pg/ml and 710.0 ±844.6 pg/ml respectively (p=0.0001) for TL patients, while IL-10 mean levels were 93.0 ±136.0 pg/ml and 32.4 ±31.7 pg/ml respectively (p= 0.0001). TST-reactive Pulmonary TB patients had significantly higher IFN-γ (851 ±234.4 pg/ml) compared to TBLNT patients (p = 0.0001), while pulmonary TB patients had significantly lower IL-10 compared to TBLNT patients (p=0.0001). Apparently healthy individuals had significantly lower IFN-γ and IL-10 levels compared to TBLNT and pulmonary TB patients (p=0.003).**

CONCLUSION: **Strong TST reactivity, high IFN-γ and IL-10 levels are good surrogate markers of active TBLNT, while increasing IFN-γ levels and decreasing IL-10 levels mark healing. Tuberculosis Skin Test reactivity although a good diagnostic marker does not disappear with treatment. WAJM 2011; 30(3): 193–196.**

Keywords: **Tuberculous lymphadenitis, cellular immune response, IFN-γ, IL-10, Purified protein derivative.**

RÉSUMÉ

CONTEXTE: Lymphadénite tuberculeuse (TL) est la forme la plus commune d'tuberculose extra-pulmonaire dans les pays tropicaux.

OBJECTIF: Cette étude visait à caractériser in vivo et in vitro les réponses immunitaires cellulaires à l'infection à Mycobacterium PPD chez les patients TL comme marqueurs de la maladie et la guérison.

MÉTHODES: Après consentement éclairé, 36 patients TL, 40 patients atteints de tuberculose pulmonaire (TB) et 20 individus apparemment sains ont été inscrits lors de leur rencontre les critères de sélection spécifiques. Le test cutané à la tuberculine (TCT) et de sang périphérique mono-nucléaires des cellules (PBMC) ont été réalisées en utilisant la culture PPD. Les cytokines ont été mesurées en utilisant des kits commerciaux.

RÉSULTATS: Le TCT moyenne était 24,6 ± 8,0 mm pour les patients TL. Le TCT a été variable chez les patients de tuberculose pulmonaire et des individus sains. Il a été réactive dans un tiers des patients atteints de tuberculose pulmonaire avec une moyenne de 20 ± 3,0 mm et réactif dans la moitié des individus en bonne santé avec une moyenne de 12,6 ± 3,2 mm. Pré niveaux moyens) γet post-traitement par l'interféron gamma (IFN- étaient 498,6 ± 905,8 pg / ml et 710,0 ± 844,6 pg / ml respectivement (p = 0,0001) pour les patients TL, tandis que l'IL-10 niveaux moyens étaient 93,0 ± 136,0 pg / ml et 32,4 ± 31,7 pg / ml respectivement (p = 0,0001). TST-réactive patients atteints de tuberculose pulmonaire avait significativement plus (851 ± 234,4 pg / ml) comparativement aux patients TBLNTγélevés d'IFN- (p = 0,0001), tandis que les patients avaient une tuberculose pulmonaire IL-10 significativement plus faible comparativement aux patients TBLNT (p = 0,0001). individus apparemment sains avaient significativement plus bas et IL-10 niveaux par rapport aux patients tuberculeux TBLNT etγd'IFN- pulmonaire (p = 0,003).

CONCLUSION: Haute et IL-10 niveaux sont bonsγla réactivité du TST Strong, l'IFN- γmarqueurs de substitution de TBLNT active, tout en augmentant l'IFN- niveaux et en diminuant l'IL-10 marque la guérison niveaux. La réactivité test de la tuberculose, bien que la peau un bon marqueur de diagnostic ne disparaît pas avec le traitement. **WAJM 2011; 30(3): 193–196.**

Mots-clés: lymphadénite tuberculeuse, la réponse immunitaire cellulaire, l'IFN- γ, IL-10, dérivé protéique purifié.

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Abbreviations: **IFN-γ,** Interferon gamma; **IL-10,** Interleukin-10; **PBMC,** Peripheral Blood Mononuclear Cell; **PPD,** Purified protein derivative; **TL,** Tuberculous lymphadenitis; **TST,** Tuberculosis skin test.

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, with 8–10 million new cases and 2–3 million deaths each year.[1] The global problem of TB is worsening with the spread of multidrug-resistant disease and increased susceptibility of HIV-infected individuals to TB.[2] The short-comings of the BCG vaccine fueled the race to identify *Mycobacterium tuberculosis* antigens suitable for the development of subunit vaccines against TB.[3–6] Host effector mechanisms that provide protection against TB are not well understood and remain an obstacle in the development of new anti-TB vaccines. In general, resistance to mycobacterial infections is mediated by interaction of antigen-specific T-cells and macrophages with production of an array of cytokines produced by these cells. Th-1 immune responses, dominated by IFN-γ secretion are the principal mediators of protective immunity against TB.[7] In addition, tumour necrosis factor-α (TNF-α), secreted by activated macrophages, contributes to anti-tuberculous action and limits disease pathology.[8,9] Th-2 responses, on the other hand are characterized by the secretion of IL-4, IL-5 and IL-10, that are associated with disease progression.[7,10] IL-10 in particular is associated with reduced resistance and chronic progressive TB.[11–13] Furthermore, it deactivates macrophages and down regulates the secretion of IFN-γ.[12] In brief, differences in the cytokine profiles could possibly determine if tuberculosis is going to resolve, progress, or become latent.[10] A number of studies were conducted to determine cytokine profiles in TB patients.[14,15] However, knowledge of the cytokine profile of human peripheral blood mono-nuclear cells (PBMCs) in response to various complex and single antigens of *M. tuberculosis* is limited. Recently, Magdorf and colleagues[16] suggested using the difference in cytokines production by PBMCs as an aid in the diagnosis of tuberculous and tuberculous lymphadenopathy.

Extra-pulmonary tuberculosis constitutes the most complicated and difficult to diagnose type of mycobacterial infection. Tuberculous lymphadenitis (TL) is the commonest type of extra-pulmonary TB especially in tropical countries.[17] In Sudan *Mycobacterium tuberculosis* is the principal cause of tuberculous lymphadenitis, while *Mycobacterium bovis* causes a rare entity of the disease.[18] This study aimed to investigate the role of the tuberculin skin test (TST) and cytokine profiles of patients with TL as possible surrogate markers of disease and healing.

SUBJECTS, MATERIALS, AND METHODS

Ethical Consideration

The study proposal was reviewed and approved by the Ethical Committee at the Institute of Endemic Diseases (IEND), University of Khartoum/Sudan. Well-trained pathologists performed fine needle aspiration (FNAC). Tuberculosis Skin Test and bleeding were done according to the Standard Operating Procedures (SOPs) of IEND.

Study Design and Study Subjects

This was a prospective and longitudinal study of two years duration. Patients with significant (palpable) lymphadenopathy (smallest diameter ≥2 cm) from different clinics and hospitals in the Sudanese capital were referred to the lymphadenopathy clinic at the Institute of Endemic Disease, University of Khartoum. Patients with pulmonary TB and healthy individuals were enrolled as comparators.

Methods

Following informed consent, clinical and demographic data were included in a pre-designed questionnaire (Table 1). The TST was performed by intradermal injection of 0.1 ml containing 5 TU PPD (Pasteur Institute, Iran) on the volar aspect of the left forearm. The test was read 48–72 hours using the ball-point pen technique.[19] An induration of 5 mm or more was taken as positive. IFN-γ and IL-10 levels were measured in culture supernatants of isolated PBMCs using commercial kits R&D systems ELISA kits (Cat. #DIF50, Germany). Purified protein derivative (PPD) at 10 µg/ml was used to stimulate PBMCs. Wells containing cell culture medium + PBMCs and cell culture medium + PBMCs + PHA at 12.5µg/ml were used as negative and positive controls, respectively. Cultures were harvested after 24 h for phyto-haemagglutinin (PHA) and 48 h for soluble PPD antigens.

RESULTS

One hundred and fifty patients with clinically significant lymphadenopathy (lymph node size ≥ 2 cm in its smallest diameter) were investigated at the clinic. Thirty-six consecutive patients with FNAC diagnosis of tuberculous lymphadenitis (TL) were enrolled in the study. The mean age of the TL patients was 31.5 ± 16.0 years (range 10–60 years). The commonest age group affected was 20–25 years. The male: female ratio was 1:1.3. The erythrocytes sedimentation rate (ESR) was found to be raised in the majority of patients (89.0%; 32/36) with a mean of 79.6 ± 25.0 mm/first hour whereas it was found to be < 30 mm in 11.1% (4/36) with a mean of 16.3 ± 6.3 mm/hour. The TST was strongly reactive (> 15 mm) in 100% of TL patients (36/36) with a mean induration of 24.6 ± 8.0 mm. The haematological profiles of the patients at diagnosis (disease) showed normal levels of haemoglobin, white cells and platelets. Following six months of treatment (healing/cure) lymph node enlargement was cleared, the hemato-logical profiles remained the same, but the ESR dropped significantly to 34.8 ± 23.3 mm/first hour (p=0.004). Forty patients with pulmonary tuberculosis and 20 apparently healthy individuals were enrolled in the study for comparison. The mean age of the patients with pulmonary disease was 32.2 ± 12.0 years (range 15–57 years). Twenty-six (65%) were non-reactive in TST; while 14(35%) were reactive with a mean induration of 20 ± 3.0 mm. The erythrocytes sedimentation rate (ESR) was high in all patients with pulmonary disease with a mean of 100.1 ± 27.4 mm/first hour. All healthy volunteers had normal haematological values and ESR of ≤20 mm/first hour.

Cytokines Profiles and TST Reactivity (Table 2)

High IFN-γ and IL-10 levels were detected in all patients with tuberculous

Table 1: Characteristics of TBLNT and PTB Patients and Healthy Controls

Variable	Mean ± SD		
	TBLNT	PTB	Control
Number	36	40	20
M : F	1 : 1.3	1 : 2	1 : 1
Age (y)	31.5 ± 16	32.3 ± 12	27.4 ± 2.6
Hb g/dl,	12.0 ± 2.7	11.5 ± 2.1	12.9 ± 1.3
WBC *l* x $10^3 mm^{-3}$,	5.5 ± 2.3	4.5 ± 2.9	5.5 ± 1.1
ESR mm/hr	69.2 ± 31.7	100.1 ± 28.0	19.3 ± 6.7
TST, tuberculosis skin testing	2224.6 ± 8.0	14.1 ± 9.3	6.2 ± 2.8

TL, Tuberculous lymphadenitis; PTB, Pulmonary tuberculosis; Controls: healthy volunteers.

Table 2: Cytokine Profiles of the Study Groups

	TL		Pulmonary TB		Healthy Individuals	
Mantoux Text	*+ve*	*–ve*	*+ve*	*–ve*	*+ve*	*–ve*
IFN-γ Level (pg/ml)						
Diagnosis	498.6 ± 905.8	–	851.0 ± 234.4	20.0 ± 18.7	31.8 ± 45.3	15.5 ± 11.1
Healing	710.0 ± 844.8	–	ND	ND	NA	NA
	p=0.0001					
IL-10 Level (pg/ml)						
Diagnosis	93.0 ± 136.0	–	26.0 ± 13.6	49.0 ± 17.9	12.4 ± 16.0	00
Healing	32.4 ± 31.7	–	ND	ND	NA	NA
	p=0.0001					

**ND, Data Not Available. NA, Not Applicable; TL, Tuberculosis lymphadenitis.*

lymphadenitis at diagnosis with a mean of 498.6 ± 905.8 pg/ml (range 0–2585) and IL-10 with a mean of 93.0 ± 136.0 pg/ml (range 0-460). At the end of the treatment (healing), IFN-γ increased significantly (mean 710.0 ± 844.6 pg/ml) compared to levels at diagnosis (p=0.0001), whereas the mean level of IL-10 decreased significantly (32.4 ± 31.7 pg/ml) compared to the level at diagnosis (diseases) (p= 0.000). The TST induration was significantly associated with higher level of IFN-γ (p=0.01). Tuberculous lymphadenitis patients had higher levels of IL-10 compared to TST-reactive healthy individuals (p=0.003). IL-10 levels were high in patients with pulmonary tuberculosis but with levels that were significantly lower than the initial levels of patients with tuberculous lymphadenitis (p=0.001). All tuberculous lymphadenitis and a third of patients with pulmonary tuberculosis who had reactive TST showed significantly higher levels of IFN-γ compared to patients with pulmonary tuberculosis and non reactive TST (p=0001). Levels of IL-10 were comparable in healthy controls with reactive TST and treated tuberculous lymphadenitis patients (p=0.7).

DISCUSSION

The increase in the number of patients with tuberculosis in the HIV/AIDS era showed clearly that TB is an opportunistic disease of poor immunity and poor communities. The role of BCG vaccine in protection against pulmonary tuberculosis and reduction in non-tuberculous mortality has been a subject of an endless debate. Now there is accumulating data that BCG most probably offers protection against tuberculous meningitis and possibly leprosy in children. Markers of immunity against tuberculosis are not well characterized; the TST does not correlate well with protection. The test is strongly positive in almost all patients with tuberculous lymphadenitis and some patients with pulmonary disease. The TST is invariably negative in patients with miliary tuberculosis and some patients with pulmonary disease. IFN-γ correlates positively with the TST induration in some patients with pulmonary tuberculosis. The fact that the TST induration does not correlate well with protection from tuberculosis has been known for some time. This could probably be explained by the fact that, testing for immunity is done in the skin while infection is usually in the alveolar spaces of the lungs. Reaction of the alveolar macrophages to mycobacterial antigens could exactly reflect patient's immune status.

In this work we demonstrated that patients with tuberculous lymphadenitis, invariably have strong *in vivo* and *in vitro* reactions to mycobacterial antigens, which is in agreement with other studies. The cytokines profile at diagnosis (disease) was a mixture of Th1 and Th2 responses as evidenced by the increase in both IFN-γ and IL-10 levels. The cytokines profile changed from a Th1/Th2 to a predominantly Th1 with persistence/increase of IFN-γ and concomitant decrease in IL-10 (healing). This is very similar to what happens in patients with post kala-azar dermal leishmaniasis (PKDL) –a dermatosis that complicates visceral leishmaniasis- who mostly have an initial mixed Th1/Th2 response in their skins which changes to a pure Th1 response following treatment.[20, 21]

The TST remained positive following successful treatment of TL patients probably indicating long lasting memory or latent infection. Patients with pulmonary tuberculosis who were included for comparison, had a variable cytokines profile, but it was evident that patient reactive in TST had the highest level of IFN-γ production (Th1). Patients with pulmonary disease and a non-reactive TST had higher levels of IL-10, with low levels of IFN-γ, indicating a predominant Th2 type of immune response. Healthy volunteers with

reactive TST had a predominantly Th1 type of response with high levels of IFN-ã and low levels of IL-10 i.e. similar to healed/cured patients with tuberculous lymphadenitis. TST non-reactive volunteers on the other hand had low levels of IFN-γ and low levels of IL-10, may indicate immune system naivety or anergy to mycobacterial antigens.

A Th1/Th2 mixed immune response is a good marker of disease, while a Th1 response (high IFN-γ and reduced IL-10) is a good marker of healing/cure. The shift from a mixed Th1/Th2 to a pure Th1 response is brought about by the treatment that probably helps to eliminate the mycobacterium and so reduce the amount of antigens presented to T-cells by macrophages. This reduction of antigen presentation will help naïve T cells to differentiate down the line of Th1 response and production of IFN-γ.

Conclusion

TST reactivity is a good surrogate marker of activity in patients with tuberculous lymphadenitis that correlates well with IFN-γ production, but not with protection. Th1/Th2 immune responses are good markers of disease, while Th1 responses correlate to healing. Conversion in TST reactivity is probably accompanied by IFN-γ production in patients with healing pulmonary tuberculosis.

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