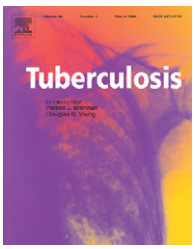
Tuberculosis (2008) **88**, 58–63

Available at www.sciencedirect.com

ScienceDirect

journal homepage: http://intl.elsevierhealth.com/journals/tube



Association between sputum smear status and local immune responses at the site of disease in HIV-infected patients with pulmonary tuberculosis

Henry C. Mwandumba[a,b,c,*], S. Bertel Squire[c], Sarah A. White[a,e], Mukanthu H. Nyirenda[a], Samuel D. Kampondeni[d], Elizabeth R. Rhoades[f], Eduard E. Zijlstra[b], Malcolm E. Molyneux[a,b,c], David G. Russell[f]

[a]*Malawi-Liverpool-Wellcome Trust Clinical Research Programme, College of Medicine, Box 30096, Blantyre 3, Malawi*
[b]*Department of Medicine, College of Medicine, Private Bag 360, Blantyre 3, Malawi*
[c]*Clinical Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*
[d]*Department of Radiology, Queen Elizabeth Central Hospital, P.O. Box 95, Blantyre, Malawi*
[e]*Centre for Medical Statistics and Health Evaluation, University of Liverpool, Liverpool L69 3BX, UK*
[f]*Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

Received 3 March 2007; received in revised form 15 June 2007; accepted 27 June 2007

KEYWORDS
Bronchoalveolar lavage;
Cellular response;
Cytokine response;
HIV;
Pulmonary TB;
Sputum smear status

Summary
Infection with human immunodeficiency virus (HIV) may affect the clinical presentation of pulmonary tuberculosis (TB). To investigate the association between sputum smear status at presentation and local pulmonary immune responses in HIV-infected patients with pulmonary TB, we compared the cellular and cytokine profiles in bronchoalveolar lavage (BAL) fluid obtained from the site of lung disease in 22 sputum smear- and culture-positive, and 17 sputum smear-negative but culture-positive pulmonary TB patients. Smear-positive patients had significantly higher BAL fluid concentrations of IL-6 ($p = 0.007$), IL-8 ($p = 0.02$), IL-10 ($p = 0.03$) and IFN-γ ($p = 0.008$) than smear-negative patients. No significant differences in the proportions of examined BAL cells were found. We concluded that sputum smear-positive TB was associated with greater pro-inflammatory and immunomodulatory cytokine responses at the site of lung disease than sputum smear-negative disease. The local immune responses may affect the clinical presentation of active pulmonary TB in HIV-infected patients.

*Corresponding author. Department of Pharmacology & Therapeutics, Sherrington Buildings, Room 2-63, University of Liverpool, Ashton Street, Liverpool L69 3GE, UK. Tel.: +44 151 794 5550; fax: +44 151 794 5540.
E-mail addresses: H.C.Mwandumba@liverpool.ac.uk (H.C. Mwandumba), S.B.Squire@liverpool.ac.uk (S. Bertel Squire), swhite@mlw.medcol.mw (S.A. White), m.nyirenda02@yahoo.co.uk (M.H. Nyirenda), err23@cornell.edu (E.R. Rhoades), eezijlstra@malawi.net (E.E. Zijlstra), mmolyneux@malawi.net (M.E. Molyneux), dgr8@cornell.edu (D.G. Russell).

1472-9792/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tube.2007.06.003

Introduction

Infection with human immunodeficiency virus (HIV) may alter the clinical presentation of active pulmonary tuberculosis (TB). During early HIV infection when immune function is relatively intact, sputum smear-positive TB predominates. In contrast, patients with advanced HIV disease and significant immunosuppression often present with sputum smear-negative and disseminated TB.[1–4] Although the correlation between sputum smear status and the degree of systemic immunity (using peripheral blood $CD4^+$ T lymphocyte count as a surrogate marker) in HIV-infected, pulmonary TB patients is well documented, the relationship between sputum smear status and local immune responses at the site of lung disease is unclear.

Previous studies which recruited predominantly HIV-negative patients with pulmonary TB reported significant differences in the types of cellular and cytokine responses in bronchoalveolar lavage (BAL) fluid obtained from the site of lung disease between patients with sputum smear-positive and/or cavitary pulmonary TB and those with sputum smear-negative and/or non-cavitary disease.[5–8] These studies suggested that the local immune responses in the lung may affect the clinical and radiological presentation of active pulmonary TB. In the present study, we tested this hypothesis in HIV-infected patients with microbiologically confirmed pulmonary TB by comparing the cellular and cytokine profiles in BAL fluid obtained from the site of lung disease between patients with sputum smear- and culture-positive TB, and those with sputum smear-negative but culture-positive disease.

Methods

Study population

Participants were recruited prospectively from the Queen Elizabeth Central Hospital in Blantyre, Malawi. They were a subset of a cohort of adults with chronic cough who underwent bronchoscopy and BAL as part of investigations to determine the cause of their respiratory symptoms. The study was approved by the Research Ethics Committees of the College of Medicine, Malawi, the Liverpool School of Tropical Medicine, UK, and Cornell University, USA. Written, informed consent to participate in the study was obtained from all participants.

Collection and laboratory processing of study samples

A sputum sample for TB diagnosis was obtained from each participant at recruitment and bronchoscopy was performed the next morning, at which a further sputum sample was obtained immediately before the procedure. In all cases, bronchoscopy was performed before commencing anti-TB chemotherapy. Sputum smears were examined by light microscopy for acid-fast bacilli (AFB) using Ziehl–Neelsen stain and cultured for mycobacteria on Löwenstein–Jensen (L–J) medium according to standard guidelines.[9] Cultures were incubated at 37 °C and examined weekly for up to 12 weeks. TB was confirmed if sputum cultures grew mycobacteria of the *Mycobacterium tuberculosis* (Mtb) complex as identified by growth characteristics and colony morphology. Bronchoscopy and BAL were performed as previously described.[10] BAL fluid was retrieved from the radiographically abnormal lung segments by gentle aspiration, pooled, and filtered through a single layer of sterile gauze. The pooled fluid was then centrifuged at 250*g* for 5 min at 4 °C; the cell-free BAL fluid was collected by gentle aspiration and stored in aliquots at −80 °C for later cytokine analysis. The cell pellet was re-suspended in RPMI-1640 and total cell count performed using a Neubauer haemocytometer. A differential cell count was obtained by counting 100 BAL cells on cytospin slides (Cytospin 3, Shandon, UK) stained with Wright's stain. All participants were offered HIV counseling and HIV testing was performed as previously described.[10] Full blood count (FBC) and peripheral blood $CD4^+$ T lymphocyte counts were determined using automated analyzers (Onyx Coulter®, Beckman Coulter, and BD FACSCount, respectively). Chest radiographs were interpreted by a radiologist (SDK).

Determination of BAL fluid cytokine and albumin concentrations

BAL fluid cytokine concentrations were determined using cytometric bead array (CBA) assay (Becton Dickinson). Frozen aliquots of cell-free BAL fluid were thawed to room temperature and concentrations of IL-1β, IL-6, IL-8, IL-10, IFN-γ and TNF-α were measured using CBA kits (Becton Dickinson) according to the manufacturer's recommendations. Standards were prepared by performing serial dilutions of concentrated bulk standard prepared as recommended by the manufacturer. Fifty microlitres of diluted standards and BAL fluid samples were added to tubes containing fluorochrome- and antibody-conjugated beads and were acquired on a FACSCalibur® flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Data were acquired and analyzed using BD CBA and BD CellQuest software (Becton Dickinson), respectively. The sensitivity limits of the assay for the measured cytokines were: IL-1β, 7.2 pg/ml; IL-6, 2.5 pg/ml; IL-8, 3.6 pg/ml; IL-10, 2.8 pg/ml, IFN-γ, 7.1 pg/ml and TNF-α, 2.8 pg/ml. Since precise quantification of cytokines recovered in BAL fluid is difficult due to mixing of normal saline used for performing the lavage and an unknown volume of epithelial lining fluid (ELF) present in the pulmonary airways, BAL fluid cytokine concentrations were standardized by expressing the data as ratios of BAL fluid albumin concentrations as previously described by Tsao and colleagues.[7] Briefly, the crude BAL fluid cytokine concentrations determined by CBA assay (pg/ml) were divided by the BAL fluid albumin concentrations (g/ml) and the results were reported as the quantity of cytokine per gram of albumin (pg/g albumin). BAL fluid albumin concentrations were determined using bromocresol green colorimetric technique according to manufacturer's recommendation (Plasmatec, UK).

Statistical analyses

Data were analyzed using Intercooled Stata software version 9.0 (Stata Corporation). Two-sample *t*-tests and Wilcoxon's