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Soluble and cell-associated triggering receptor expressed on myeloid cells-1 and -2 in patients with pulmonary tuberculosis

Dear Editor,

Recently, Huang and colleagues reported in this Journal that serum levels of soluble Triggering Receptor Expressed on Myeloid Cells (TREM)-1 are elevated in Taiwanese patients with pulmonary tuberculosis (TB).[1] We here expand these data and report on soluble and cell-associated TREM-1 and TREM-2 in patients with lung TB in Bangladesh.

TB remains a disease with a major impact on global health with an estimated one-third of the world population infected with *Mycobacterium (M.) tuberculosis* and a attributed mortality rate of 1.7 million deaths a year.[2] Bangladesh is a highly affected country with 225 new cases per 100,000 population in 2012 (World Health Organization, Global Tuberculosis Report 2013). TREM-1 has been described as a transmembrane receptor that can amplify signaling through pattern recognition receptors, while TREM-2 is regarded as a negative modulatory of innate inflammatory signaling.[3,4] TREM-1 and TREM-2 also exist in soluble forms; whereas soluble TREM-1 has been studied as a potential diagnostic marker in especially respiratory tract infections, reports on soluble TREM-2 levels in human disease are limited.[3,4] Studies on the expression of TREM-1 and -2 in TB are of interest considering their role in Toll-like receptor (TLR) signaling, which comprise important components of host defense during infection with the causative agent *M. tuberculosis*.[5] The aim of the current study was to determine soluble TREM-1 and TREM-2 levels in serum, and membrane-bound TREM-1 and TREM-2 on peripheral blood mononuclear cells of patients with pulmonary TB.

57 patients and 31 healthy blood donors (≥18 years) were recruited in the Tuberculosis Clinic of Chittagong General Hospital and the Chittagong Medical College & Hospital, Chittagong, Bangladesh. These subjects were part of a larger population in which the expression of Toll-like receptor (TLR) regulators was studied.[6] In- and exclusion criteria have been reported in detail.[6] On-site TB confirmation was defined by a minimum of two out of three positive Ziehl—Neelsen stained sputum samples collected on two consecutive days. *Mycobacterium tuberculosis* infection was confirmed by polymerase chain reaction (GeneXpert, Cepheid, Solna, Sweden). All patients were tested for human immunodeficiency virus infection by a Determine® HIV 1/2 test (Alere, Tilburg, The Netherlands). The study was approved by the National Research Ethics Committee (NREC), Bangladesh Medical Research Council, Bangladesh and the Oxford Tropical Research Ethics Committee, University of Oxford, Oxford, UK (OXTREC 35-09). Written informed consent was obtained from all study subjects or next-of-kin by a native Bengali speaker. Peripheral blood mononuclear cells (PBMCs) were isolated from 18 TB patients and 16 healthy controls using Cell Preparation Tubes (Becton Dickinson, Franklin Lakes, NJ). PBMCs were frozen in RPMI 1640 (Life technologies) medium with 20% FCS (Lonza, Basel, Switzerland) and 20% DMSO with a Mr. Frosty freezing container (Thermo Scientific, Waltham, MA) and stored in liquid nitrogen until use. Prior to analysis stored cells were carefully thawed, washed and stained with the following antibodies: CD14-APC-Cy7, CD3-Alexa Fluor 700, CD4-PE (all BD Biosciences, San Jose, CA), CD8-PE-Cy7 (eBioscience, Vienna, Austria), TREM-1-PERCP (R&D Systems, Minneapolis, MN) and TREM-2-APC (R&D Systems) for 30 min at 4 °C. Data acquisition was performed using a FACSCanto II (BD Biosciences) flow cytometer. Geometric mean fluorescence intensities (MFIs) were corrected with

Table 1 Patients and healthy controls.

	Cell-associated TREM-1/2			Soluble TREM-1/2		
	Healthy controls	Tuberculosis	p value	Healthy controls	Tuberculosis	p value
n	16	18		31	57	
Age (years)	28 ± 5.9	29 ± 9.1	0.99	30 ± 5.6	32 ± 13	0.7328
Sex (male/female)	13/3	12/6	0.449	23/8	42/15	1.000
Smoker	4 (25%)	10 (56%)	0.09	9 (29%)	28 (49%)	0.079
Fever (n, %)	0 (0%)	18 (100%)	<0.0001	0 (0%)	57 (100%)	<0.0001
Night sweats (n, %)	0 (0%)	5 (28%)	0.046	0 (0%)	22 (39%)	<0.0001
Weight loss (n, %)	0 (0%)	13 (72%)	<0.0001	0 (0%)	42 (74%)	<0.0001
Fatigue (n, %)	0 (0%)	8 (44%)	0.0031	1 (3%)	32 (56%)	<0.0001
Shortness of breath (n, %)	0 (0%)	2 (11%)	0.487	0 (0%)	7 (12%)	0.0445
Productive cough (n, %)	3 (19%)	18 (100%)	<0.0001	2 (6%)	55 (96%)	<0.0001
Temperature (°C)	36.7 ± 0.3	37.4 ± 0.9	0.0172	36.8 ± 0.5	37.7 ± 0.9	<0.0001
Heart rate (beats/minute)	80.6 ± 6.1	92.5 ± 14.6	0.0073	81.0 ± 6.0	92.2 ± 15.3	<0.0001
Respiratory rate (breaths/minute)	20.0 ± 2.6	25.7 ± 3.8	<0.0001	21.4 ± 3.6	26.7 ± 5.9	<0.0001
Body mass index (w/l^2)	24.8 ± 3.6	18.1 ± 4.5	<0.0001	24.4 ± 3.4	17.7 ± 3.1	<0.0001

N: total number. Data are represented as mean ± SD or n (%). p values were determined by Mann Whitney U tests or Fisher's exacts test where applicable compared to healthy donors.

fluorescence minus one (FMO) controls, negative values were set to 0 MFI. Levels of soluble TREM-1 and TREM-2 were measured in serum of 57 TB patients and 31 healthy controls by enzyme-linked immunosorbent assays (TREM-1: R&D systems, Abingdon, UK; TREM-2: Sino Biological Inc., Beijing, China). Detection limits were: TREM-1: 31.25 pg/ml and TREM-2: 46.87 pg/ml. Comparisons between groups were performed using Mann—Whitney U tests or by Fisher's exact test with GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA). $p < 0.05$ was considered statistically significant.

Table 1 shows the demographic and clinical characteristics of patients and healthy blood donors. Consistent with the report by Huang et al.,[1] patients with TB had elevated soluble TREM-1 levels (Fig. 1, panel A, $p < 0.01$ versus healthy donors). Blood monocytes of TB patients displayed

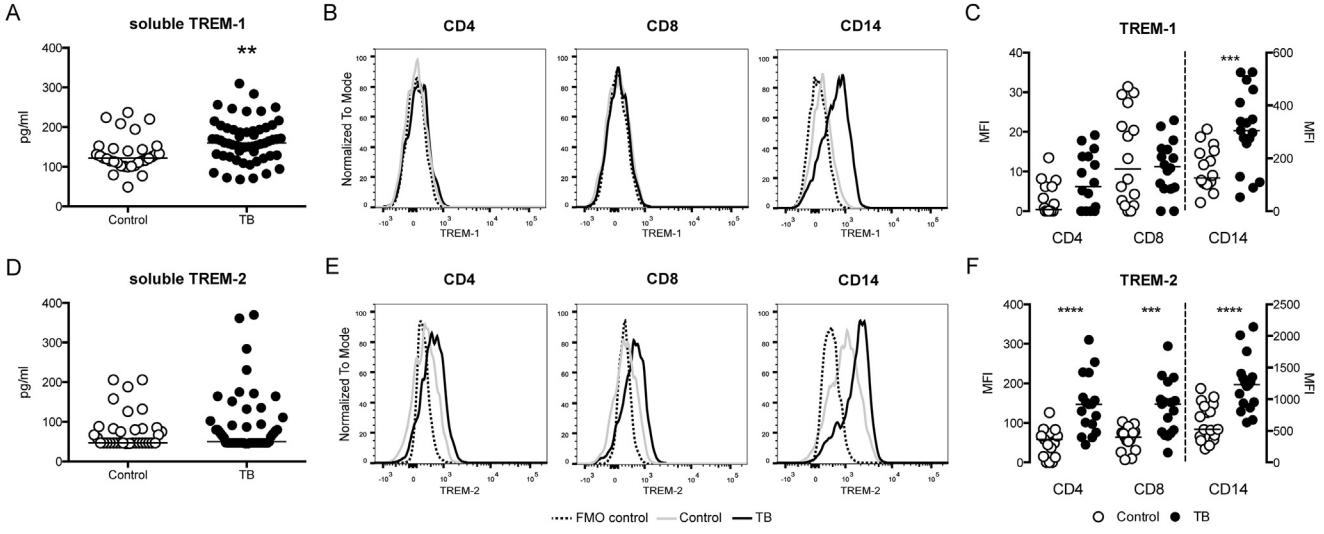

Figure 1 Serum levels of soluble TREM-1 and -2, and expression of cell-associated TREM-1 and -2 in patients with pulmonary tuberculosis and healthy blood donors. Serum concentrations of soluble TREM-1 **(A)** and soluble TREM-2 **(D)** in healthy blood donors (n = 31) and TB patients (n = 57). Data are represented as individual measurements with medians. TB: tuberculosis. Histograms showing flow cytometric analysis and corresponding dot plots of TREM-1 **(B, C)** and TREM-2 **(E, F)** on CD4 T-cells, CD8 T-cells and CD14 monocytes of healthy blood donors (grey lines or open symbols, n = 16) and patients with TB (black lines or closed symbols, n = 18). Representative histograms are shown. Fluorescence minus one (FMO) controls are shown as dotted lines. Data are represented as individual measurements with medians. TB: tuberculosis; FMO: Fluorescence minus one. MFI: geometric mean fluorescence intensity. **p < 0.01, ***p < 0.001, ****p < 0.0001.