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Decrease in $CD4^+CD25^+FoxP3^+$ T_{reg} cells after pulmonary resection in the treatment of cavity multidrug-resistant tuberculosis

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SUMMARY

Objectives: Immune regulatory mechanisms may limit the immunopathologic condition of infection with *Mycobacterium tuberculosis* and suppress cellular immune responses in the host. We investigated the $CD4^+CD25^+FoxP3^+$ circulating regulatory T cells (T_{reg}) in patients with cavity multidrug-resistant tuberculosis (MDR-TB) before and after surgery.

Methods: We compared the proportion of T_{reg} cells in 13 patients with cavity MDR-TB pre- and postoperatively and in 10 healthy control subjects by flow cytometry using three specific markers in peripheral blood lymphocytes: cell-surface CD4 and CD25 expression and intracellular FoxP3 expression.

Results: The proportion of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} was significantly higher in patients with cavity MDR-TB and at 1-month postoperatively than in healthy controls ($p < 0.001$). The proportion of $CD4^+$ and $CD4^+CD25^-$ cells was significantly lower in patients with cavity MDR-TB than in controls ($p < 0.001$). Pre- and postoperative proportions of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} cells showed a positive correlation ($r = 0.878$, $p < 0.001$).

Conclusion: Circulating T_{reg} cells are increased in proportion in patients with cavity MDR-TB and decreased after surgery. Infection with *M. tuberculosis* may induce T_{reg} cell-surface molecular changes with increased numbers of cells.

1. Introduction

Tuberculosis (TB) is associated with chronic, persistent antigen stimulation in vivo to maintain a sustained immune response, which suppresses but generally fails to eradicate *Mycobacterium tuberculosis* (MTB) infection. Worldwide, an estimated 9.27 million cases of TB occurred in 2007.[1] Strains of *M. tuberculosis* that are resistant to both isoniazid and rifampin with or without resistance to other drugs have been termed multidrug-resistant TB (MDR-TB). Drug resistance information from 114 countries and the two special administrative regions (SARs) of China (i.e., Hong Kong and Macau), combined with nine epidemiological factors, revealed the global proportion of MDR-TB among new and previously treated cases to be 4.8% (95% confidence interval 4.6–6.0%). China and India carry approximately 50% of the global burden of disease.[2] The proportion of MDR-TB is higher than before, but the reasons are unclear. Host genetic factors may contribute to the development of MDR-TB, and incomplete and inadequate treatment is the most important factor leading to its development. MDR-TB is difficult to control by medical therapy alone, and surgery has emerged as a therapeutic option.[3,4]

$CD4^+$ and $CD8^+$ T-cell-mediated immunity is the main protection against TB.[5] $CD4^+$ regulatory T cells (T_{reg} cells) play a key role in the control of the immune system. The most commonly used marker for T_{reg} cells is CD25 (interleukin 2 (IL-2) receptor α),[6] although the recently identified transcription factor FoxP3 is more specific.[7] The suppression function of FoxP3 mRNA expression is mediated via direct cell–cell contact or the secretion of interleukin 10 (IL-10) or transforming growth factor beta (TGF-β).[8] The proportion of $CD4^+CD25^{high}$ T cells and levels of FoxP3 mRNA expression have both been found to be significantly higher in peripheral blood mononuclear cells (PBMCs) of patients with TB than in cells of healthy controls.[9] Also, the proportion of $CD4^+CD25^{high}$ T cells and level of $CD4^+CD25^{high}$ FoxP3 mRNA have been found to be significantly higher in PBMCs of patients with active TB than in cells of patients with latent *M. tuberculosis*

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Table 1
Indication (therapeutic vs. diagnostic) and main clinical pathology when therapeutic.

Subject	Sex	Age	Chest CT	TST[a]	Sputum MTB		Clinical feature			Anti-TB drug therapy (years)	Resection type
					Smear	Culture	Fever	Cough (years)	Hemoptysis		
1	M	29	(1) Type III TB; (2) RLL cavity concurrent infection	+++	+++	+	37.5–39.0 °C	Cough 3 years intermittently	–	2.5	Wedge resection
2	M	53	(1) Type III TB; (2) LUL cavity	++	++	+	37.3–38.8 °C	Cough 7 years intermittently	+	6	Wedge resection
3	M	18	(1) Type III TB; (2) LUL cavity concurrent infection	+++	+	+	37.0–39.0 °C	Cough 1.5 years intermittently	–	1	Lobectomy
4	M	53	(1) Type III TB; (2) RUL cavity concurrent aspergilloma	++	–	+	37.5–38.5 °C	Cough 5 years intermittently	–	4.5	Lobectomy
5	M	70	(1) Type III TB; (2) RUL cavity concurrent aspergilloma	++	+	+	37.2–38.6 °C	Cough 6 years intermittently	+	5.5	Lobectomy
6	M	42	(1) Type III TB; (2) RLL cavity concurrent aspergilloma	++	+	+	37.5–39.5 °C	Cough 10 years intermittently	+	7	Lobectomy
7	F	59	(1) Type III TB; (2) RUL cavity concurrent infection	++	+	+	37.5–39.0 °C	Cough 20 years intermittently	+	7	Lobectomy
8	F	52	(1) Type III TB; (2) LUL cavity concurrent aspergilloma	++	–	+	37.2–38.5 °C	Cough 5 years intermittently	+	3.5	Wedge resection
9	M	57	(1) Type III TB; (2) LUL cavity concurrent infection	++	+	+	37.5–39.5 °C	Cough 4 years intermittently	–	3	Lobectomy
10	M	53	(1) Type III TB; (2) RUL cavity concurrent aspergilloma; (3) RCW abscess	++	+	+	37.2–39.5 °C	Cough 3.5 years intermittently	–	3	Lobectomy
11	F	51	(1) Type III TB; (2) RL destroyed	++	+	+	37.4–39.6 °C	Cough 20 years intermittently	+	15	Pulmonary resection
12	M	49	(1) Type III TB; (2) LL destroyed	+++	++	+	37.6–38.9 °C	Cough 7 years intermittently	+	5.5	Pulmonary resection
13	F	62	(1) Type III TB; (2) LUL cavity concurrent aspergilloma; (3) RCW abscess	++	+	+	37.2–38.9 °C	Cough 11 years intermittently	–	9.5	Lobectomy

CT, computerized tomographic scanning; TST, tuberculin skin test; MTB, *Mycobacterium tuberculosis*; TB, tuberculosis; RLL, right lower lobe; LUL, left upper lobe; RUL, right upper lobe; RCW, right chest wall; RL, right lobe; LL, left lobe.

[a] TST (PPD 5 U 0.1 ml, measure the red spot after 72 h): negative –: <5 mm; positive +: 5–9 mm; ++: 10–19 mm; +++: ≥20 mm.

Table 2
Bacterial susceptibility test results of patients preoperatively.

Subject	Sex	Age	Susceptibility test results							
			SM	INH	RFP	EMB	LVX	D	CTH-1321	CPM
1	M	29	R	R	R	R	S	S	S	S
2	M	53	S	R	R	S	R	R	S	S
3	M	18	S	R	R	S	S	S	S	S
4	M	53	R	R	R	R	R	S	S	S
5	M	70	S	R	R	S	R	R	S	S
6	M	42	R	S	R	S	S	S	S	S
7	F	59	R	R	S	S	S	S	S	S
8	F	52	S	R	S	R	S	S	S	S
9	M	57	R	R	R	R	S	S	S	S
10	M	53	R	R	R	S	R	S	S	S
11	F	51	R	R	R	R	R	R	S	S
12	M	49	R	R	R	R	R	S	S	S
13	F	62	S	R	R	S	S	S	S	S

SM, streptomycin; INH, isoniazid ; RFP, rifampin; EMB, ethambutol; LVX, levofloxacin ; D, dipasic (pasiniazid); CTH-1321, prothionamide; CPM, capreomycin; R, resistant; S, sensitive.

infection or in controls.[10] In contrast, the proportion of $CD4^+CD25^{high}$ $FoxP3^+$ cells is not increased,[11] nor is the proportion of $CD4^+CD25^{high}$ T cells decreased[12] ex vivo in patients with active TB disease.

Because immunopathology is thought to play a major role in the pathogenesis of active TB, $CD4^+CD25^{high}$ T_{reg} cells are considered part of the regulatory mechanisms of immunity on invasion by *M. tuberculosis*. Thus, we aimed to determine whether elevated T_{reg} cell numbers may be decreased after pulmonary resection in patients with pulmonary MDR-TB and found that the relative increase in T_{reg} cell ratios is significantly decreased after pulmonary resection.

2. Methods

2.1. Patients and controls

Between January 2006 and December 2008, we enrolled 13 patients (nine men; mean age 50 years, range 18–70 years) undergoing surgery for pulmonary tuberculosis at The Third People's Hospital of Shantou City. These patients exhibited cavity MDR-TB concurrent infection ($n = 5$), cavity MDR-TB concurrent aspergilloma ($n = 6$), or lung destruction ($n = 2$). The mean time with anti-tubercular drug therapy was 5.6 years (range 1–15 years). The indication (therapeutic vs. diagnostic) and the main clinical pathologies are shown in Table 1. Results of bacterial susceptibility testing are shown in Table 2. The main clinical symptom status of patients at postoperative month 6 (POM6) is shown in Table 3. In subject 4, the right upper lobe cavity showed concurrent aspergilloma (Figure 1, A1–A4), and the patient underwent lobectomy of the right lung in October 2008. Subject 11 showed a destroyed right lobe and underwent pulmonary resection in September 2006 (Figure 2, B1–B5).

Peripheral blood was obtained from patients before and 1 and 6 months after surgery. We recruited 10 healthy volunteers (seven men; mean age 42 years, range 25–63 years). All patients underwent postoperative individualized chemotherapy to ensure long-term cure. All subjects were HIV-negative. The study protocol was approved by our institutional review board for human studies, and informed consent was obtained from all subjects.

Table 3
Main clinical symptom status of patients at postoperative month 6.

Subject	Sex	Age	Chest CT (comparison with preoperative)	Sputum MTB		Clinical features		
				Smear	Culture	Body temperature	Cough	Hemoptysis
1	M	29	RLL cavity removal	–	–	36.9 °C	No cough	–
2	M	53	LUL cavity removal	–	–	36.7 °C	Cough occasionally	–
3	M	18	LUL cavity removal	–	NE	36.8 °C	No cough	–
4	M	53	RUL cavity and aspergilloma removal, residue intrapulmonary air containing space with thick wall	–	–	36.7 °C	Cough intermittently	–
5	M	70	RUL cavity and aspergilloma removal, residue intrapulmonary air containing space with thick wall	–	NE	36.5 °C	Cough occasionally	–
6	M	42	RLL cavity and aspergilloma removal, residue intrapulmonary air containing space with thick wall	–	NE	36.9 °C	NR	–
7	F	59	RUL cavity removal	–	NE	36.4 °C	Cough occasionally	–
8	F	52	LUL cavity and aspergilloma removal	–	–	36.5 °C	Cough occasionally	–
9	M	57	LUL cavity removal	–	–	36.8 °C	Cough occasionally	–
10	M	53	RUL cavity and aspergilloma, and RCW abscess removal, pleura thickening	–	NE	36.5 °C	Cough intermittently	–
11	F	51	RL removal, residue intrapulmonary air containing space with thick wall	–	–	36.8 °C	Cough intermittently	–
12	M	49	LL removal, residue intrapulmonary air containing space with thick wall	–	NE	36.8 °C	Cough intermittently	–
13	F	62	LUL cavity and aspergilloma and RCW abscess removal, pleura thickening	–	–	36.5 °C	Cough intermittently	–

CT, computerized tomographic scanning; MTB, *Mycobacterium tuberculosis*; RLL, right lower lobe; LUL, left upper lobe; RUL, right upper lobe, RCW, right chest wall; RL, right lobe; LL, left lobe; NE, no examination; NR, no record.

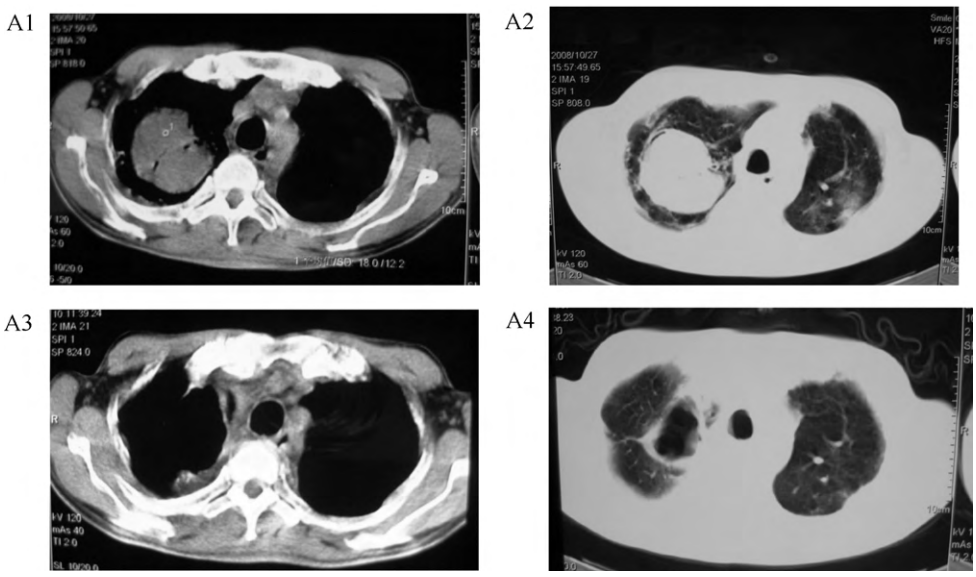

Figure 1. Computed tomography (CT) scan of a 53-year-old man with multidrug-resistant tuberculosis. Preoperative CT scan of the chest in October 2008 (A1, A2): right upper lung cavity shows concurrent aspergilloma. Postoperative CT scan of the chest in April 2009 (postoperative month 6: A3, A4): focus of infection was removed and residual intrapulmonary air-containing space with thick wall.

2.2. Blood sample preparation

Whole blood was collected in 12 × 75-mm plastic tubes containing anti-coagulant (EDTA-K2) and immediately underwent cellular staining. Whole blood (100 μl) was aliquoted (per tube) along with 20 μl of appropriate test antibody or respective isotype control for three color tests: fluorescein isothiocyanate (FITC) anti-FoxP3 (clone PCH101, CAT.11-4766, eBioscience, San Diego, CA, USA), phycoerythrin peridinin chlorophyll protein (PerCP) anti-CD4 (clone RPA-T4, CAT.300528, Biolegend, San Diego, CA, USA), and phycoerythrin (PE) anti-CD25 (clone B1.49.9, CAT.IM0479u, Beckman Coulter, Los Angeles, CA, USA). The following isotype control antibodies were used: FITC rat IgG2b (κ, CAT.11-4031, eBioscience), PerCP mouse IgG1 (clone MOPC-21, CAT.400145, Biolegend), PE mouse IgG1 (clone 679.1 Mc7, CAT.IM0670u, Beckman Coulter). After surface staining for 30 min in the dark at room temperature, erythrocytes were lysed, and cells were fixed with the use of immuno-prep reagents (Beckman Coulter) using a Q-prep Immunology workstation (Beckman Coulter). Surface-stained cells then underwent intracellular FoxP3 staining with use of the anti-FoxP3 staining kit according to the manufacturer's recommendations.

2.3. Data acquisition and analysis

Listmode data were acquired on the Epics XL flow cytometer (Beckman Coulter) and were analyzed using EXPO32 software (Beckman Coulter). The lymphocyte gate was generated by use of forward and side-angle scattered (FSC/SSC) light window leukogating to analyze the cell-surface antigens CD4 and CD25. To determine the proportion of T_{reg} cells, $CD4^+$ T cells were gated by plotting forward versus side scatter to analyze the intracytoplasm antigen against FoxP3.

2.4. Statistical analysis

Values are presented as mean ± standard deviation (SD). All results were analyzed using SPSS v 13.0 (SPSS Inc., Chicago, IL, USA). Differences in means between patients and controls were analyzed by

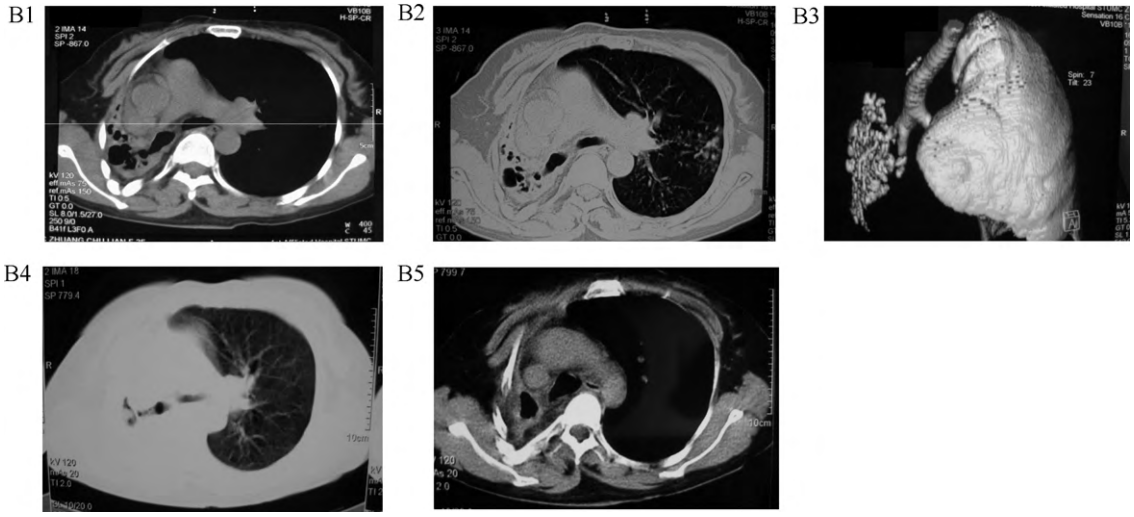

Figure 2. Computed tomography (CT) scan of a 51-year-old female with multidrug-resistant tuberculosis. Preoperative CT scan of the chest in September 2006 (B1, B2, B3): right lung destroyed and collapsed, the left lung showing scale-up complications. Postoperative CT scan of the chest in May 2007 (postoperative month 6: B4, B5): focus of infection was removed and residual intrapulmonary air-containing space with thick wall.

one-way analysis of variance (ANOVA); differences in means for patients pre- and postoperatively were analyzed by paired-samples *t*-test and correlation was examined by Spearman rank correlation analysis. A *p*-value of <0.05 was considered statistically significant.

3. Results

To determine the frequency of T_{reg} cells, gated lymphocytes and $CD4^+$ cells were analyzed for expression of CD4, CD25

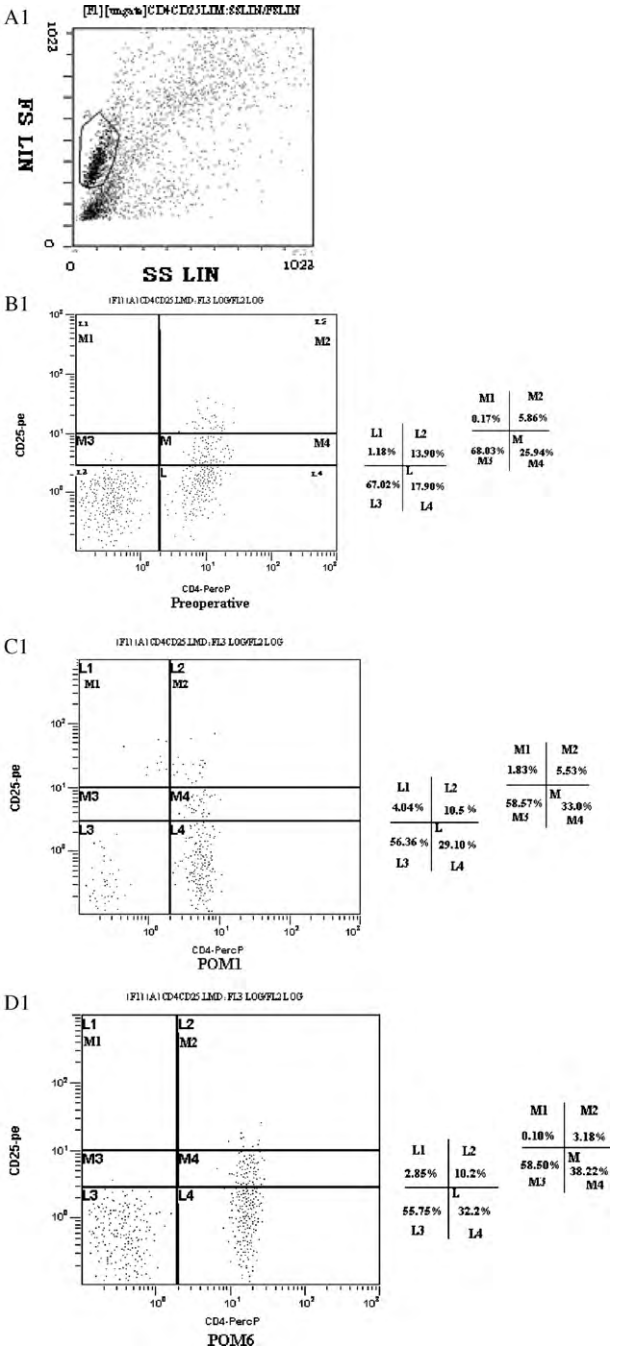

Figure 3. Flow cytometry analyses of $CD25^{+high}$ expression by $CD4^+$ T cells. The lymphocyte gate was generated by use of forward and side-angle scattered light window (FSC/SSC) dot plots (A1). Representative flow cytometry results of surface CD4 and CD25 expression on peripheral blood lymphocytes from a patient with active TB preoperatively (B1), at postoperative month 1 (POM1; C1), and at postoperative month 6 (POM6; D1). L1: $CD4^-CD25^+$ cells; L2: $CD4^+CD25^+$ cells; L3: $CD4^-CD25^-$ cells; L4: $CD4^-CD25^+$ cells; M1: $CD4^-CD25^{+high}$ cells; M2: $CD4^+CD25^{+high}$ cells; M3: $CD4^-CD25^{-high}$ cells; M4: $CD4^-CD25^+$ cells.

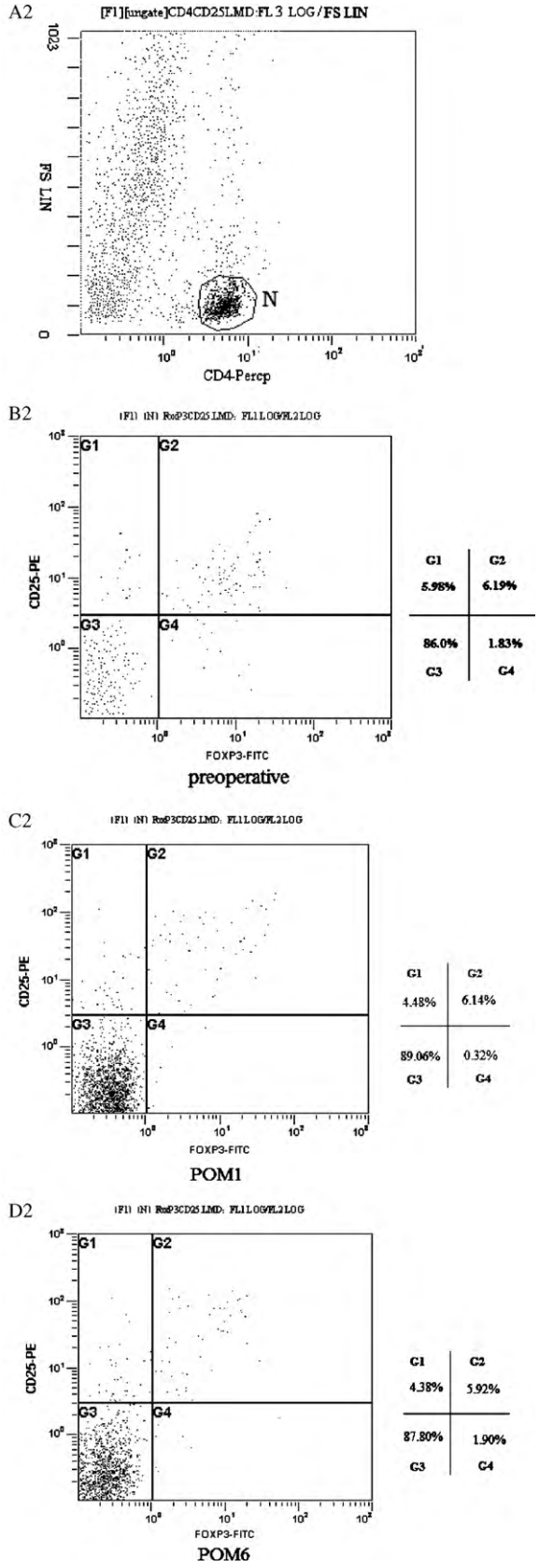

Figure 4. Flow cytometry analysis of intracellular FoxP3 and surface CD25 expression on $CD4^+$ T cells in patients with tuberculosis (TB). The $CD4^+$ T cells are shown gated (A2). Representative flow cytometry results of intracellular FoxP3 and surface CD25 expression on peripheral blood lymphocytes from a patient with active TB preoperatively (B2), at postoperative month 1 (POM1; C2), and at postoperative month 6 (POM6; D2). G1:$CD4^+CD25^+FoxP3^-$ cells; G2:$CD4^+CD25^+FoxP3^+$ cells; G3:$CD4^+CD25^-$ $FoxP3^-$ cells; G4:$CD4^+CD25^-$ $FoxP3^+$ cells.

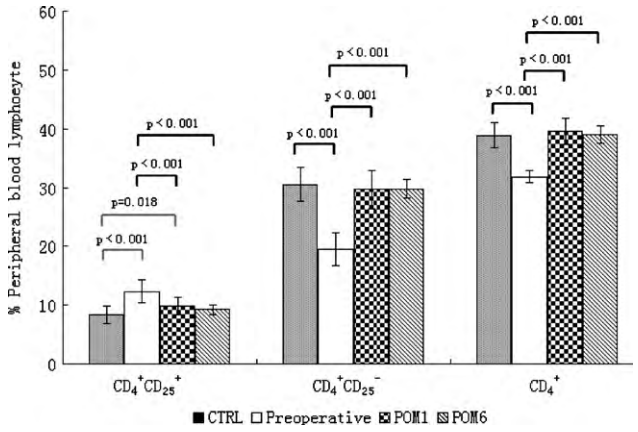

Figure 5. Percentage of $CD4^+$ cells, $CD4^+CD25^+$ cells, and $CD4^+CD25^-$ cells in non-infected control subjects (CTRL; $n = 10$), and in patients with cavity MDR-TB ($n = 13$) pre- and postoperatively: preoperative, postoperative month 1 (POM1), and postoperative month 6 (POM6).

(representative plots in Figure 3, A1–D1), and FoxP3 (representative plots in Figure 4, A2–D2).

3.1. Phenotypic analysis of $CD4^+$ cells in patients with cavity MDR-TB pre- and postoperatively

On flow cytometry, as compared with healthy controls, patients with MDR-TB showed significantly decreased proportions of $CD4^+$ T cells preoperatively ($31.85 \pm 1.03\%$ vs. $38.91 \pm 2.10\%$); this was increased postoperatively compared with preoperatively (postoperative month 1 (POM1) $39.59 \pm 2.26\%$, postoperative month 6 (POM6) $39.02 \pm 1.47\%$) ($p < 0.001$). Also, the proportion of $CD4^+CD25^+$ cells was significantly increased preoperatively ($12.28 \pm 1.96\%$ vs. $8.36 \pm 1.54\%$ for controls) and decreased postoperatively compared with preoperatively (POM1 $9.91 \pm 1.50\%$, POM6 $9.23 \pm 0.78\%$) ($p < 0.001$). However, the proportion of $CD4^+CD25^-$ cells was significantly decreased preoperatively ($19.56 \pm 2.78\%$ vs. $30.55 \pm 2.82\%$ for controls) and increased postoperatively compared with preoperatively (POM1 $29.68 \pm 3.26\%$, POM6 $29.78 \pm 1.56\%$) ($p < 0.001$) (Figure 5).

3.2. The frequency of $CD4^+CD25^{high}$ T_{reg} cells was increased in patients with cavity MDR-TB before surgery

The frequency of $CD4^+CD25^{high}$ T_{reg} cells was determined on flow cytometry. The lymphocytes were gated to analyze $CD4^+CD25^{high}$ T_{reg} cells. As compared with controls, patients with MDR-TB showed significantly increased proportions of $CD4^+CD25^{high}$ T_{reg} cells both preoperatively and at 1-month postoperatively ($7.00 \pm 1.10\%$, POM1 $6.25 \pm 1.23\%$ vs. $4.16 \pm 1.02\%$ for controls) ($p < 0.001$), with no significant increase in proportion of $CD4^+CD25^{high}$ T_{reg} cells at 6-months postoperatively compared with healthy controls ($p > 0.05$) (Figure 6).

3.3. The proportion of $CD25^+FoxP3^+$ T_{reg} cells in $CD4^+$ cells was high in patients preoperatively

Since FoxP3 transcription factor is considered the most reliable molecular marker of $CD4^+CD25^{high}$ T_{reg} cells, we investigated the intracellular expression of FoxP3 in $CD4^+CD25^+$ T cells. FoxP3 expression was significantly higher in patients preoperatively and at 1-month postoperatively as compared with healthy controls ($7.45 \pm 1.19\%$, POM1 $6.74 \pm 1.03\%$ vs. $4.50 \pm 1.03\%$) ($p < 0.001$). However, at 6-months postoperatively, the proportion of $CD4^+CD25^+FoxP3^+$ T_{reg} cells was similar between healthy subjects and patients ($p > 0.05$) (Figure 7).

3.4. The proportion of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} cells decreased at 6-months postoperatively

We investigated the proportion of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} cells pre- and postoperatively. The proportion of both $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} cells in patients with TB was significantly lower at 6-months postoperatively than preoperatively ($p < 0.001$), with no significant difference between preoperative and 1-month postoperative proportions ($p > 0.05$) (Figures 8 and 9).

3.5. Correlation between the expression of $CD4^+CD25^{high}$ cells and $CD4^+CD25^+FoxP3^+$ cells in MDR-TB patients

We analyzed the correlation between the proportion of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ cells in peripheral blood in patients with active cavity MDR-TB and found a significant

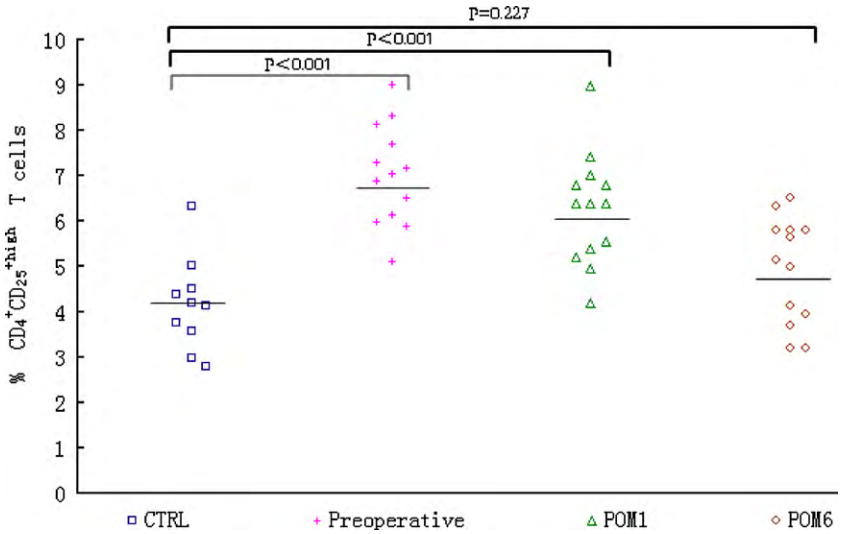

Figure 6. The proportion of $CD4^+CD25^+$ cells that are $CD25^{high}$ in non-infected controls (CTRL; $n = 10$) and patients with cavity MDR-TB before and after surgery ($n = 13$): preoperative, postoperative month 1 (POM1), and postoperative month 6 (POM6). Horizontal bars indicate median values.

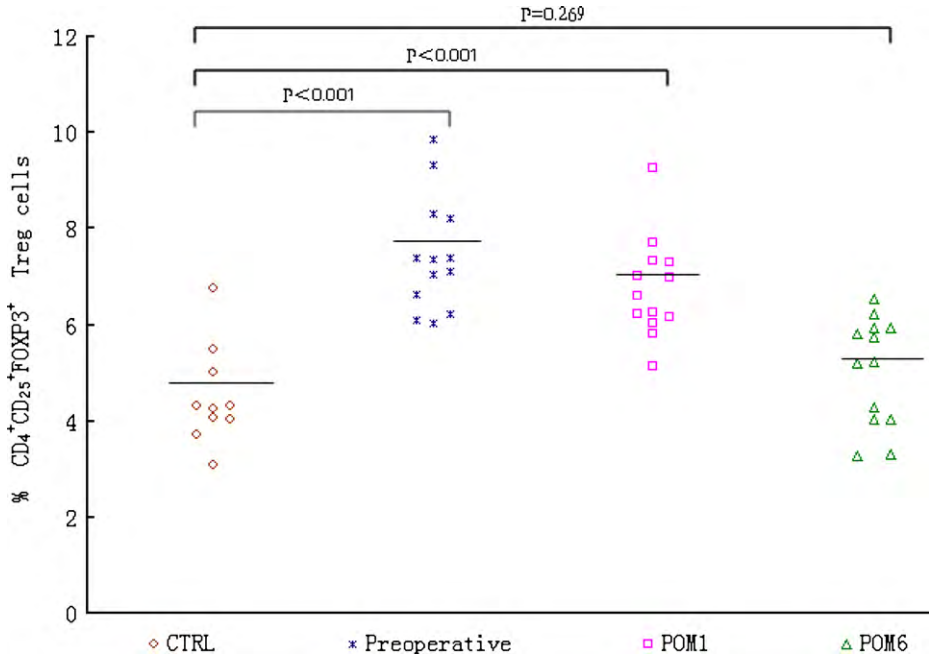

Figure 7. The proportion of $CD4^+CD25^+FoxP3^+$ T_{reg} cells among the $CD4^+$ cells measured by flow cytometry in 10 non-infected control subjects (CTRL) and 13 patients with cavity MDR-TB pre- and postoperatively: preoperative, postoperative month 1 (POM1), and postoperative month 6 (POM6). Horizontal bars indicate median values.

correlation ($r = 0.878$; $p < 0.001$; using data from all subjects) (Figure 10).

4. Discussion

The estimated worldwide incidence of TB and MDR-TB continues to increase. Despite the success of medical therapy alone, resistance to drugs and complications of the disease still present a challenge.[13] The host response to infection with *M. tuberculosis* involves the cellular immune system of T-helper 1-type interferon-γ-secreting CD4 and CD8 effector T cells.[14] This response helps to limit bacterial replication and dissemination in vivo and causes important immunopathologic features such as inflammation, but in general fails to eradicate the infection. We suggested that the immune system has regulatory mechanisms to suppress the effector response to persistent antigens. Indeed, we found the proportion of $CD4^+CD25^{high}$ and $CD4^+CD25^+FoxP3^+$ T_{reg} cells significantly higher in patients with cavity MDR-TB and at 1-month postoperatively than in healthy controls. Also, the

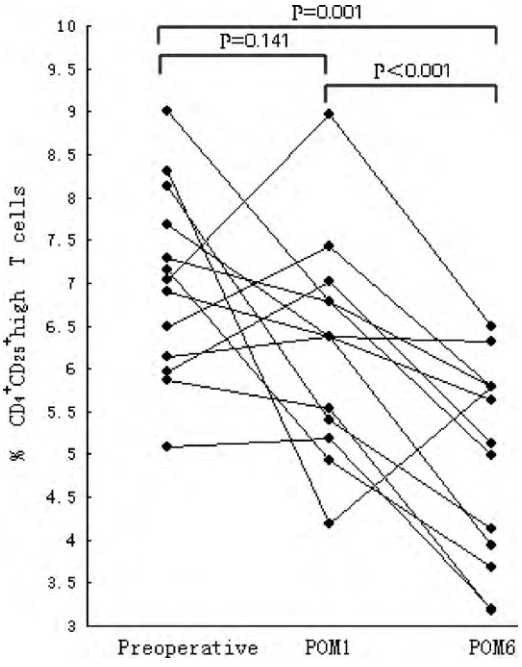

Figure 8. The proportion of the $CD4^+CD25^+$ cells that are $CD25^{high}$ in patients with cavity MDR-TB pre- and postoperatively ($n = 13$): preoperative, postoperative month 1 (POM1), and postoperative month 6 (POM6).

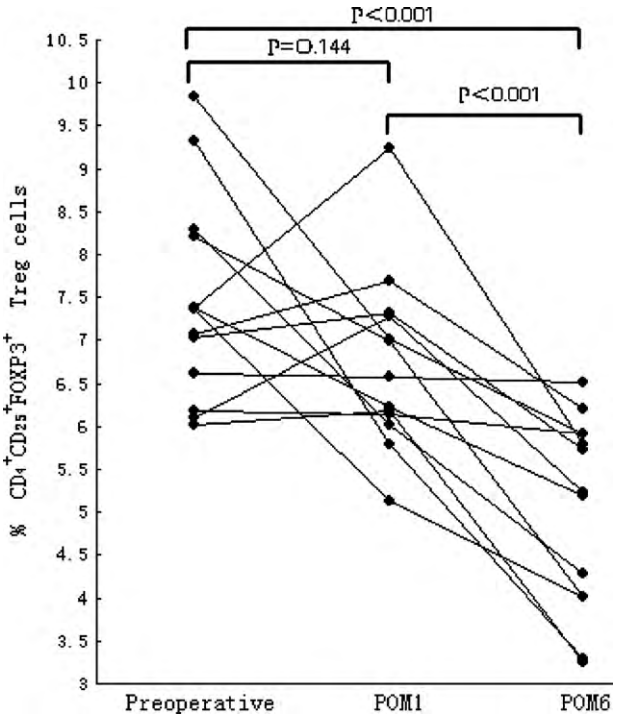

Figure 9. The proportion of the $CD4^+CD25^+FoxP3^+$cells in patients with cavity MDR-TB pre- and postoperatively ($n = 13$): preoperative, postoperative month 1 (POM1), and postoperative month 6 (POM6).

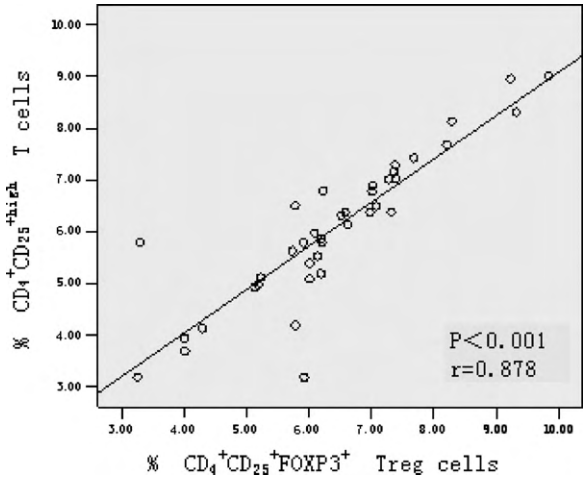

Figure 10. Correlation between the proportion of $CD4^+CD25^{+high}$ cells and $CD4^+CD25^+FoxP3^+$ cells ($r = 0.878$, $p < 0.001$).

proportion of $CD4^+$ and $CD4^+CD25^-$ cells was significantly lower in patients with cavity MDR-TB than in healthy controls.

Increasingly, FoxP3-expressing T_{reg} cells are considered critical for suppressing immune responses to self-antigens and preventing autoimmunity and for regulating immunity to foreign antigens, especially those derived from pathogens that establish persistent infections.[15] T_{reg} cells were found to prevent eradication of tubercle bacilli by suppressing an otherwise efficient $CD4^+$ T-cell response.[16] To investigate T_{reg} cells, we used the markers $CD4^+$ and high levels of cell-surface CD25 expression and intracellular FoxP3 expression. The expression of $CD4^+CD25^{high}$ cells and $CD4^+CD25^+FoxP3^+$ cells was significantly increased in peripheral blood of patients with cavity MDR-TB, which is in agreement with the report by Guyot-Revol et al.[9] Previously, the proportion of $CD4^+CD25^{high}$ T cells and $CD4^+CD25^{high}$ FoxP3 cells was found significantly higher in PBMCs of patients with active TB as compared with those with latent TB infection and controls, with no difference between those with latent infection and healthy controls.[10] However, whether T_{reg} cells decreased in number after cure of active TB was unknown. Therefore, we analyzed the expression of $CD4^+CD25^{high}$ cells and $CD4^+CD25^+FoxP3^+$ cells at 1 and 6 months after surgery in patients with cavity MDR-TB. The proportion of $CD4^+CD25^{high}$ cells and $CD4^+CD25^+FoxP3^+$ cells was significantly decreased in peripheral blood at 6-months postoperatively as compared with preoperatively and 1-month postoperatively, and did not significantly differ from that in healthy controls. One possible mechanism for T_{reg} cells suppressing anti-*M. tuberculosis* immunity is that $CD4^+CD25^+FoxP3^+$ regulatory T cells produce the cytokine TGF-β and/or IL-10, which depresses interferon-gamma (IFN-γ) production.[17] Neutralization of TGF-β and IL-10 each result in an increase in elicited IFN-γ.[18] $CD4^+CD25^+FoxP3^+$ T_{reg} expansion in TB patients may contribute to suppress *M. tuberculosis* immune responses by inhibiting IFN-γ production of PBMCs in patients with active TB or pleural fluid mononuclear cells in patients with tuberculous pleurisy.[19] However, the mechanisms by which T_{reg} cells increase in number are incompletely understood and need further research. The bacterial burden may, in part, induce the increase in T_{reg} cells.

MDR-TB is caused by *M. tuberculosis* strains resistant to at least rifampin and isoniazid. Because failure of medical treatment leads to a dismal prognosis, surgical resection has been advocated to supplement medical therapy in some patients with tracheal or bronchial stenosis, aspergillomas growing on cavitated lesions, bronchiectasis, destroyed lungs, or massive hemoptysis. Our patients showed a good prognosis after surgery, which suggests that the immunity function recovered after surgery. Because morbidity and mortality rates are acceptable, surgical intervention can be considered safe and effective in patients with pulmonary TB.

Conflict of interest

No conflict of interest to declare.

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