Poster #819

Treg/Th17 and T-cell effector responses in Tuberculosis patients co-infected with HIV

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BACKGROUND

Whether the balance between Effector and Regulatory T cell (Treg) responses against Mycobacterial Tuberculosis (MTb) may impact the outcome of clinical status of Tuberculosis (TB) infection has not been fully explored. Moreover, how these responses are perturbed and correlate with the clinical status of TB in patients co-infected with HIV is unknown.

The objectives of this study were 1) to explore the specificity and the functional profile of memory Th17 and Treg populations (peripheral Treg and Thelper frequencies in PBMC, as well as suppressive activity of Treg on MTb-specific responses) in TB patients either co-infected or not with HIV-1 and 2) to determine whether these immune parameters were correlated to the clinical status of the TB infection.
Comparisons of these immunological parameters were performed in: i) non HIV-infected TB+ patients versus healthy donors (HD), ii) non HIV-infected TB+ versus TB+ HIV+ co-infected patients, and iii) TB+ patients depending on the presence or not of sputum bacillary load (SBL).

METHODS

- *Ex vivo* frequency of Treg ($CD25^{high}CD127^{low}FoxP3+$) and their ecto-enzyme CD39 expression, as well as Th1/Th2/Th17 cells frequencies among CD4+ T cells were analyzed by flow cytometry from a cohort of TB patients, co-infected or not with HIV (n=11 TB+ HIV+; n=25 TB+ HIV-) and healthy donors (n=16 HD). The differential expression of surface markers CCR4, CCR6 and CXCR3 on memory CD4+ T cells (CD4+ CD45RA-) allowed the characterization of T cell subsets : Th1 (CCR4- CCR6- CXCR3+), Th2 (CCR4+ CCR6- CXCR3-) and Th17 (CCR4+ CCR6+ CXCR3-).
- Treg function was assessed by coculture of Treg-enriched PBMC from 9 HD (magnetic beads from Miltenyi) with autologous PBMC stimulated with PPD with or without anti-CD39 blocking Ab (4 subjects) allowing the analysis of the role of CD39 enzyme on Treg suppressive function. The frequency of PPD-specific CD4+ T cells producing IFNγ, TNFα and/or IL17A/IL17F was measured.
- MTb-specific responses were detected after 2-or 5-day stimulation of PBMC from HD and TB+ patients with PPD and/or ESAT6-CFP10 by measuring cytokine levels in culture supernatants (Multiplex assays, 15-plex from Millipore: IFNγ, TNFα, IL2, IL5, IL9, IL10, IL13, IL17A, IL17F, IL22, IL27, GM-CSF, IL1β, IL23p19, CCL20). Only significant differences in cytokine levels are shown.

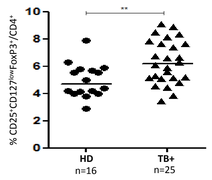
PATIENTS

- 57 TB patients enrolled in 4 sites (Créteil, Paris, Bobigny)
- 15 women (26.3%)
- Age (mean ±SD) : 40.2 years ± 16.1
- 18/57 co-infected with HIV+ (32%)
  - 7/18 on HAART
  - CD4 T cell count (mean±SD) : 181 ± 172/mm3
  - HIV-1 viral load (median) : 5.4 log_{10}
- Data of sputum bacillary load (2 ND)
  - n=31 with (SBL+)
  - n=24 without (SBL-)
- Pulmonary localisation in the majority of patients (for 16 patients: LN, peritoneal, pleural, hepatic, splenic, urinary)

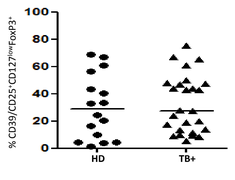
RESULTS

Comparison of TB+ infected patients and healthy subjects

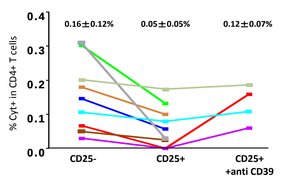
Ex vivo Treg frequency



Expression of CD39 on Treg



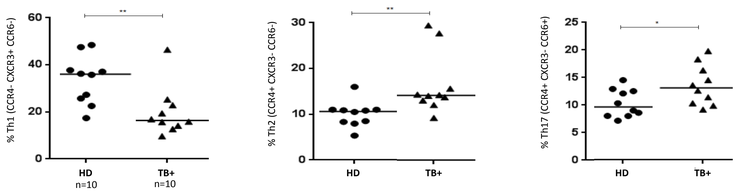
Treg function



➢ Increase of Treg in TB+ patients

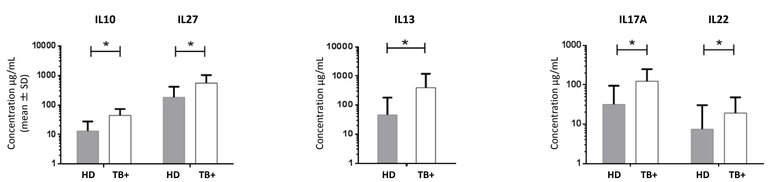
➢ Partial reversion of Treg suppressive effect on CD4+ T cell response by anti-CD39 Ab

Ex vivo Thelper frequency: expression of CCR4, CXCR3 and CCR6 on CD4+CD45RA-



➢ Increase of Th2 and Th17 frequencies and decrease of Th1 frequency in TB+ patients

Cytokine levels in SN of PPD-stimulated PBMC for 2 and 5 days (Mutiplex assay, 15-plex)

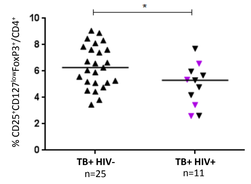


In TB+ patients:

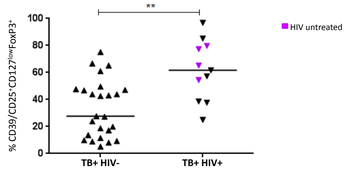
➢ Increase of Treg cytokines ➢ Increase of Th2 cytokines ➢ Increase of Th17 cytokines

Impact of HIV infection on TB infected patients : Comparison of TB+ infected and TB+ HIV+ co-infected patients

Ex vivo Treg frequency

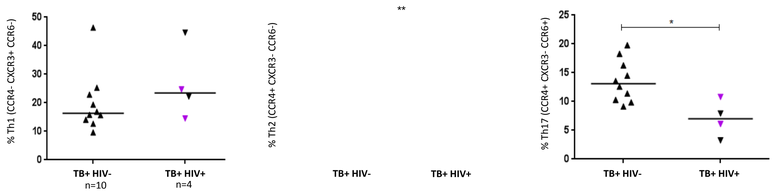


Expression of CD39 on Treg



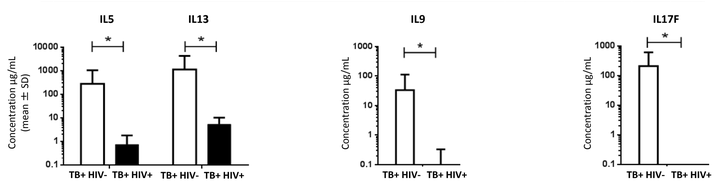
➢ Decrease of Treg in co-infected TB+ HIV+ patients, but higher CD39 expression

Ex vivo Thelper frequency: expression of CCR4, CXCR3 and CCR6 on CD4+CD45RA-



➢ Decrease of Th2 and Th17 frequencies in TB+ HIV+ patients

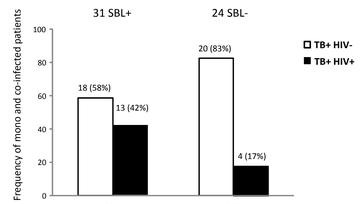
Cytokine levels in SN of ESAT6-stimulated PBMC for 2 and 5 days (Mutiplex assay, 15-plex)



In co-infected patients:

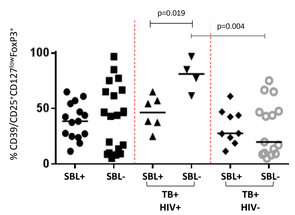
➢ Decrease of Th2 cytokines ➢ Decrease of Th9 cytokines ➢ Decrease of Th17 cytokines

Impact of the presence of sputum bacillary load : Comparison of TB+ patients with (SBL+) and without (SBL-) SBL



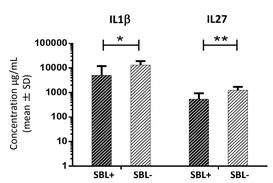
➢ Higher frequency of TB mono-infected patients among smear negative patients

Expression of CD39 on Treg



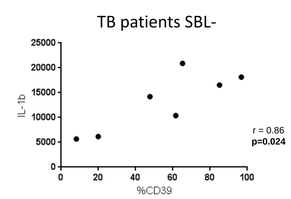
➢ Higher expression of CD39 by Treg in co-infected smear negative patients

Cytokine levels (SN of ESAT6-stimulated PBMC)



➢ Smear negative TB patients exhibit a higher MTb-specific response in terms of IL1β and IL27

TB patients SBL-



➢ In smear negative TB patients, correlation between Treg function and secretion of IL1β

CONCLUSION

- TB infection, characterized by an increased frequency of peripheral Treg and a MTb response-biased towards Th2/Th17, is impacted in patients co-infected with HIV who exhibit a lower Th2 and Th17 cell frequencies and related cytokines.
- Patients without sputum bacilli, more frequently found in mono-infected TB+ compared to co-infected TB+ HIV+ patients, are characterized by a higher IL1β and IL27 levels in response to TB antigens, which may represent a signature of TB infection control in the pulmonary site.
- This study suggests a role of IL1β/IL27 in mycobacterial clearance. Correlation between the ATP/adenosine pathway and production of IL27 and pro-inflammatory cytokines in responses to MTb antigens is under investigation.

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Acknowledgements

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- ✓ All study volunteers