232. New developments in the immunological diagnosis of tuberculosis and latent tuberculosis infection

1870 Different polyfunctional characteristics of RD1-specific CD4+ T-cells in active TB disease and LTBI
Elsa Petruccioli1, Linda Pietroni2, Valentina Vanini2, Gilda Cuzzi1, Francesco Nicola Laura3, Gisella Goletti4, 1Translational Research Unit, Department of Epidemiology and Preclinical Research, “L. Spallanzani” National Institute for Infectious Diseases-INMI, IRCCS, Rome, Italy; 2Clinical Department, “L. Spallanzani” National Institute for Infectious Diseases-INMI, IRCCS, Rome, Italy

Introduction: CD4+ T-cells and their cytokines are crucial for protection against M. tuberculosis (Mtb). Analyses of the cytokines coexpressed by polyfunctional T-cells can help in discriminating different tuberculosis (TB) stages.

Aims: To evaluate by flow cytometry the functional status and phenotype of Mtb-specific CD4+ T-cells in TB subjects at different stages.

Methods: We enrolled 25 TB patients before and after therapy (active/past TB) and 39 latent TB infection (LTBI), classified as recent/remote infection and LTBI post-prophylaxis. We evaluated the CD4+ intracelullar cytokines production (IFN-γ, TNF-α, IL-2) and memory/effector status after in vitro whole blood stimulation with RD1 antigens.

Results: Magnitude of CD4+ T-cells is higher in active TB compared to the other groups, although the differences are not significant. Double IFN-γ+/TNF-α+ CD4+ T-cells are significantly higher in active TB than in past TB (p=0.03) and in LTBI (p=0.002), whereas triple IFN-γ+/TNF-α+/IL-2+ are significantly associated to LTBI post-prophylaxis compared to active TB (p=0.02). The proportion of total IFN-γ+ CD4+ T-cells increases whereas the proportion of total IL-2+ CD4+ T-cells decreases in active TB compared to LTBI (p=0.02). Effector memory CD4+ T-cells are significantly higher in active TB than in LTBI (p<0.01), whereas central memory cells are higher in LTBI than active TB (p=0.03).

Conclusions: Double IFN-γ+/TNF-α+ CD4+ T-cells are associated to active TB disease whereas triple polyfunctional cells are associated to infection containment. These results may be helpful for better characterizing TB immune responses and generating tools for TB stages identification.

1871 Screening for latent TB using antigen-specific IP-10 response and the effect of prednisolone treatment on IP-10 responses
Erikka Belard1, Synne Semb2, Anne Marie Weiland3, Renée Solberg4, 1Rheumatology, University Hospital, Hvidovre, Denmark; 2Johannes Molde University College, Molde, Norway; 3Copenhagen HIV Program CHIMP University, Copenhagen, Denmark; 4Dept. Gastroenterology, University Hospital, Hvidovre, Denmark

Background: IGRA are recommended tests in the screening for latent tuberculosis infection in patients with autoimmune diseases before anti-TNF-α treatment.

The aim of this study was to evaluate the performance of IP-10 as an alternative biomarker to IFN-γ.

Method: Blood samples from 79 patients with Rheumatoid Arthritis and 63 patients with Inflammatory Bowel Diseases were stimulated using the QFT-IT tubes. IFN-γ and IP-10 were measured in the supernatant with ELISA.

Results: Overall the performance of IP-10 was comparable to IFN-γ (agreement 130/142, 96.5% kappa 0.80) with high levels of IP-10. QFT-IT and IP-10 test results were positive in 3(2.3%) and 4 (3.2%) patients respectively reflecting the low incidence in Denmark. Prednisonolone treated patients had significantly more indeterminate QFT and IP-10 test results: 28%(10/36) and 22% (8/36) compared to patients receiving other DMARDs 2%(2/106) and 0% (0/106) respectively.

This effect was dose dependent for both tests (p<0.0001 test for trend)

Similarly, the median IFN-γ and IP-10 responses to mitogen were reduced in prednisolone treated patients (2.81 (IQR 0.05–3.10) and 7.8 ng/ml (IQR 2.3–16.7) respectively) compared to patients receiving other DMARDs (>100IU/ml) (IQR 8.43–10.00) and 20.9 (14.5–25.8ng/ml) (p<0.0001) respectively.

228 2323s

Conclusion: This is the first study to investigate the influence of prednisolone treatment on IP-10 responses; IP-10 was equally affected by prednisolone and the study was too small to determine the value of a combined biomarker approach. IP-10 can be stored on filter paper bypassing centrifugation, freezer and cold chain which gives an IP-10 based test an advantage to the current IFN-γ based test.

1872 Latent tuberculosis infection is associated with increased Tregs frequencies in the BAL of healthy contact persons
Christian Herzmann1, Martin Ernst2, Stefan Ehlers3, Steffen Stenger4, Jörgen Maertzdorf5, Giovanni Sottoli6, Christoph Lange3, 1Clinical Infectious Diseases, Research Center, Borstel, Germany; 2Division of Immune Cell Analytics, Research Center, Borstel, Germany; 3Molecular Immunology Medicine, Research Center, Borstel, Germany; 4Institute for Medical Microbiology and Hygiene, University Hospital, Ulm, Germany; 5Department of Immunology, Max Planck Institute for Infection Biology, Berlin, Germany; 6Clinical Epidemiology and Medical Statistics Unit, Sassari University, Sassari, Italy

Background: Only a minority of individuals exposed to Mycobacterium tuberculosis (MTB) develops a positive Interferon-γ release assay (IGRA) from peripheral blood mononuclear cells (PBMC). Most contact persons do not develop a systemic immune response after inhalation of MTB aerosols. Pulmonary immune mechanisms preventing a systemic immune response in these subjects are incompletely understood.

Objective: To evaluate local and systemic immune responses in healthy MTB contacts.

Methods: Recruitment of MTB exposed health care workers (HCWs) and very close private house hold contacts (HHCs). Flow cytometry of bronchoalveolar lavage (BAL) cells and PBMC for immunophenotyping. IGRA testing of blood and BAL cells.

Results: 35 HCWS and 15 HHCs were recruited. Regulatory Tregs (CD4+CD25+CD127-) frequencies were increased in all contacts with latent MTB infection (PBMC IGRA positive; n=15) compared to contacts with a negative PBMC IGRA (n=25) with a median 2.12%, IQR 1.63-3.01 versus 0.68%; p=0.003. No immunophenotypic differences were seen in PBMC between IGRA positive and IGRA negative subjects (9.6%; 5.9-10.1 versus 7.7%; 4.6-11.3; p=0.47). In 5 of the 25 IGRA negative subjects, the BAL IGRA gave a positive result, possibly indicating incipient tuberculosis.

Conclusion: In close MTB contacts with LTBI, Tregs are increased in BAL but not systemically when compared to contacts that remain IGRA negative. The functional role of Tregs requires further investigation.

1873 Evaluation of non-tuberculous mycobacteria effect in the tuberculosis infection diagnosis: Interim analysis of a TBNET study
Jose Dominguez1, Irene Latore2, Maria Tosla1, Virginia Amanatidou1, Nuria Diez2, Irene Mialdea2, Neus Altet4, Mar Serra1, Jessica Diaz1, Alicia Lacoma1, Malu De Souza5, Juan Ruiz-Manzano1, Eman Giner2, Cristina Prat1, Amparo Escobrano1, Vicente Austria1. 1Microbiology & Pneumology, Hospital Univ. Germans Trias i Pujol. CIBER Enfermedades Respiratorias, Badalona, Spain; 2Pediatric & Epidemiology, Universidad de Valencia, Spain; 3Pediatrics, University of Athens School of Medicine, Athens, Greece; 4Programa de Prevencion y Control de la Tuberculosis de Barcelona, Institut Catolà de la Salut, Barcelona, Spain

The aim was to determine the role of previous non-tuberculous mycobacteria (NTM) sensitization in children as a factor of discordant results between tuberculin skin test (TST) and T-SPOT.TB assay (Oxford Immunotec). We studied the presence of M.avium sensitized T cells in 87 non BCG vaccinated paediatric patients with discordant results: TST positive and T-SPOT.TB negative. We also included as controls 11 individuals with a negative TST and a negative T-SPOT.TB, and 8 patients with microbiologically confirmed NTM infection. Peripheral blood mononuclear cells were stimulated with M.avium sensitin. The presence of reactive T cells were determined by means of ELISPOT.

From the 87 children, in 31 cases (35.6%) we obtained a positive ELISPOT result after stimulation with M avium sensitin, in 50 cases the result was negative (57.5%), and in the remaining 6 cases the test failure. The number of responder T cells were determined by means of ELISPOT.

Abstract printing supported by Chiesi Visit Chiesi at Stand B2.10

Monday, September 3rd 2012
In line with previous studies the QFT had poor sensitivity in HIV-infected TB patients, whereas the cut-off decreased from 0.35 to 0.135 IU/ml sensitivity could be increased from 79.8% (CI: 70.3-87.4) to 87.2% (CI: 78.8-93.2) without a significant loss of specificity (specificity= 98.0% (CI: 95.8-99.3)). We were not able to demonstrate a similar effect among the HIV-infected as the increase in sensitivity occurred at a high compromise in specificity (from 98.1 to 94.3%).

In the children both tests had poor performance with low positivity rate and high indeterminate rate; this was not explained by risk factors for poor test performance. In the children the positivity rate was low in all groups for both tests. The diagnostic sensitivity improved in both HIV-negative and -positive by adjusting the cut-off for a positive test in HIV-positive individuals.

Materials and methods: This case-control study pools data from three cohorts from Tanzania and Denmark. Cases were 72 HIV-positive and 94 HIV-negative Tanzanian patients with confirmed TB. The control-groups comprised 389 HIV-negative and 305 healthy Danish high-school students with no known risk factors for TB. Potential cut-offs were determined by ROC curve analysis.

Results: HIV infected TB patients had lower responses to M tuberculosis antigens compared to non-HIV infected TB patients, median 0.9 IU/ml (IQR 0.6-1.5) vs. 1.5 IU/ml (IQR 0.6-1.5) (p=0.006). In the groups of HIV non-infected, ROC curve analysis suggested that by reducing the cut-off from 0.35 to 0.135 IU/ml sensitivity could be increased from 79.8% (CI: 70.3-87.4) to 87.2% (CI: 78.8-93.2) without a major loss of specificity (specificity=98.0% (CI: 95.8-99.3)). We were not able to demonstrate a similar effect among the HIV-infected as the increase in sensitivity occurred at a high compromise in specificity (from 98.1 to 94.3%).

Conclusion: In line with previous studies the QFT had poor sensitivity in HIV-infected. The diagnostic sensitivity improved in both HIV-negative and -positive by lowering the cut-off, but specificity was significantly compromised in HIV-positive non-exposed Danish controls compromising the benefit of this approach to improve the QFT test.