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

Elisa Petruccioli[1], Linda Petrone[1], Valentina Vanini[1], Gilda Cuzzi[1], Francesco Nicola Lauria[2], Delia Goletti[1]. *[1]Translational Research Unit, Department of Epidemiology and Preclinical Research, "L. Spallanzani" National Institute for Infectious Diseases-INMI, IRCCS, Rome, Italy; [2]Clinical 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+ intracellular cytokines production (IFNγ, TNFα, IL2) 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 IFNγ$^+$TNFα$^+$ CD4+ T-cells are significantly higher in active TB than in past TB (p=0.03) and in LTBI (p=0.002), whereas triple IFNγ$^+$TNFα$^+$IL2$^+$ are significantly associated to LTBI post-prophylaxis compared to active TB (p=0.02). The proportion of total IFNγ 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 IFNγ$^+$TNFα$^+$ 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 prednisolon

Pernille Ravn[1], Erika Belard[2], Synne Semb[2], Anne Marie Werlinrud[5], Bolette Søborg[4], Merete Lund Hetland[3], Inge Nordgaard Larsen[5], Morten Ruhwald[1]. *[1]Clinical Research Centre, University Hospital, Hvidovre, Denmark; [2]Rheumatology, University Hospital, Glostrup, Denmark; [3]Epidemiology, Staten Serum Institute, Copenhagen, Denmark; [4]Copenhagen HIV Program CHIP, University, Copenhagen, Denmark; [5]Dpt. Gastroenterology, University Hospital, Hvidovre, Denmark*

Background: IGRAs 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 138/142, (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 TB incidence in Denmark. Prednisolone 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->10) and 7.8ng/ml (IQR 2.2-16.7) respectively) compared to patients receiving other DMARDs (>10.0IU/ml (IQR 8.43->10.0) and 20.9 (14.5-25.8ng/ml) (p<0.0001))respectively.
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 Herzmann[1], Martin Ernst[2], Stefan Ehlers[3], Steffen Stenger[4], Jeroen Maertzdorf[5], Giovanni Sotgiu[6], Christoph Lange[1]. *[1]Clinical Infectious Diseases, Research Center, Borstel, Germany; [2]Division of Immune Cell Analytics, Research Center, Borstel, Germany; [3]Molecular Inflammation Medicine, Research Center, Borstel, Germany; [4]Institute for Medical Microbiology and Hygiene, University Hospital, Ulm, Germany; [5]Department of Immunology, Max Planck Institute for Infection Biology, Berlin, Germany; [6]Clinical 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%; 0.32-0.96, respectively (p<0.0001). 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 Dominguez[1], Irene Latorre[1], Maria Tsolia[3], Virginia Amanatidou[3], Nuria Diez[2], Irene Mialdea[2], Neus Altet[4], Mar Serra[1], Jessica Diaz[1], Alicia Lacoma[1], Malu De Souza[4], Juan Ruiz-Manzano[1], Empar Giner[2], Cristina Prat[1], Amparo Escribano[2], Vicente Ausina[1]. *[1]Microbiology & Pneumology, Hospital Univ. Germans Trias i Pujol. CIBER Enfermedades Respiratorias, Badalona, Spain; [2]Pediatrics & Epidemiology, Universidad de Valencia, Spain; [3]Pediatrics, University of Athens School of Medicine, Athens, Greece; [4]Programa de Prevención y Control de la Tuberculosis de Barcelona, Institut Català 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 microbiologicaly 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 after M.avium sensitin stimulation was significantly higher that the number of responder T cells after specific M.tuberculosis antigens stimulation. In all children included as controls were obtained negative ELISPOT results after stimulation with M.avium sensitin. The differences of the number of responder T cells to M.avium sensitin between the study and the control group were significant. In 4 cases a positive result was obtained between patients with confirmed NTM infection. Our results suggest that previous NTM sensitization in children induces false positive results in TST for diagnosing latent tuberculosis infection. The use of IFN-γ tests provide a more specific diagnostic of TB infection in childhood.

1874

IP10 as a diagnostic marker for childhood tuberculosis in a Tanzanian population

Line Lindebo Holm[1], Michala Vaaben Rose[2], Thomas Nørlykke[1], Pernille Ravn[3], Morten Ruhwald[1]. *[1]Clinical Research Centre, Hvidovre Hospital, Copenhagen, Denmark; [2]Dept. of Medicine, Amager Hospital, Amager, Denmark; [3]Dept. of Infectious Diseases, Odense University Hospital, Odense, Denmark*

Intro

A main challenge in childhood TB management is the lack of good diagnostic tools. IP-10 is a novel marker for latent tuberculosis (TB), but very few studies have examined the use of IP-10 in childhood TB.

Aims: To compare the performance of the Quantiferon test (QFT) and IP-10 for the diagnosis of TB in Tanzanian children

Methods: 207 TB suspected children (0-15 years) and 102 adults with confirmed TB were included. QFT tests were analyzed locally and IP-10 was measured using ELISA. The children were divided in to three risk groups using clinical parameters, CXR, microscopy and culture: Confirmed/highly probable TB (probTB, n=33), possible TB (possTB, n=84) and Not TB (notTB, n=90).

Results: In children the positivity rate was low in all groups for both tests.

Table 1

	QFT			IP-10		
	POS	NEG	INDET	POS	NEG	INDET
probTB	15%	67%	18%	21%	64%	15%
possTb	17%	54%	29%	14%	54%	32%
notTB	8%	65%	27%	7%	61%	32%

IP-10 positivity rate was associated with TB risk groups (p=0.02), QFT was not (p= 0.12). The agreement between IP-10 and QFT was 70%, discordance was mainly found in the possTB and notTB groups, combining tests did not increase test sens. For both tests indet. outcome was not associated with HIV status, young age or malnutrition.

In adults the sens. was 80% for both tests; and the indet. rate was 11% for IP-10 and 6% for the QFT (agree. 83%, k=0.5, p<0.0001).

Discussion: 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 adults the QFT and IP-10 test performance was in line with other studies. Our findings could be explained by group heterogeneity and severity of illness.

1875

Evaluation of the QuantiFERON-TB Gold in tube cut-off for diagnosing tuberculosis in HIV-infected and non-HIV infected individuals

Line Lindebo Holm[1], Martine G. Aabye[1], Pernille Ravn[2], Morten Ruhwald[1]. *[1]Clinical Research Centre, Hvidovre Hospital, Hvidove, Denmark; [2]Dept. of Infectious Medicine, Odense University Hospital, Odense, Denmark*

Background: The QuantiFERON-TB Gold In tube (QFT) is used for diagnosing tuberculosis (TB) infection. But the test has compromised sensitivity in HIV infected patients. This study assesses if the diagnostic accuracy of the QFT test can be improved by adjustment of 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-positive patients 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 0.9IU/ml (IQR 0.1-0.9) vs. 1.5IU/ml (IQR 0.6-1.5) (p<0.04). 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.

1876

Response to Mtb Rv2628 latency antigen associates with bacterial containment

Linda Petrone[1], Valentina Vanini[1], Elisa Petruccioli[1], Teresa Chiacchio[1], Gilda Cuzzi[1], Kees L. M. C. Franken[2], Francesco Nicola Lauria[3], Enrico Girardi[4], Tom H.M. Ottenhoff[2], Delia Goletti[1]. *[1]Translational Research Unit, Department of Epidemiology and Preclinical Research, National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy; [2]Department of Infectious Diseases, Leiden University Medical Center, Leiden, Netherlands; [3]Clinical Department, National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy; [4]Clinical Epidemiology Unit, Department of Epidemiology and Preclinical Research, National Institute for Infectious Diseases "Lazzaro Spallanzani", Rome, Italy*

Introduction: Interferon(IFN)γ response to Mtb Rv2628 latency antigen is associated with latent tuberculosis infection (LTBI). Immune response against Rv2628 may contribute to evaluate Mtb infection control.

Aims: To compare the Rv2628 specific response in recent contacts of patients with active TB before, during and after isoniazid prophylaxis.

Methods: In this cross-sectional study we evaluated 139 QuantiFERON TB-Gold In-Tube (QFT-IT) positive contacts: 37 enrolled at baseline, 32 during prophylaxis, 17 at the end of prophylaxis, 53 after 1 year of prophylaxis completion. Whole blood IFNγ response to Rv2628 and QFT-IT (early phase antigens) at day 1 post-culture was evaluated. Controls unexposed to Mtb were also included.

Results: IFNγ levels in response to Rv2628 antigen were significantly higher at baseline than after 1 year prophylaxis completion (p<0.0001). The quantitative IFNγ response to QFT-IT was significantly higher at baseline than at the end of prophylaxis (p=0.023) or after 1 year prophylaxis completion (p=0.001); however all the subjects were QFT-IT positive at all the time points considered. Unexposed controls did not respond to Rv2628 and QFT-IT.

Conclusions: Whole blood IFNγ response to Rv2628 antigen is significantly reduced in QFT-IT-positive contacts after 1 year prophylaxis completion. Rv2628 is considered a latency antigen therefore it is unclear at the moment if the decreasing response is due to the fact that prophylaxis allows Mtb eradication and a consequent decrease in latency responses, or whether prophylaxis inhibits Mtb to remain in a latent stage. These results may be helpful to better characterize LTBI immune response and to generate tools to monitor prophylaxis efficacy.