

**Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes**

**Project: Global Transcriptome Analysis of Pulmonary Lesions from Tuberculosis Patients**

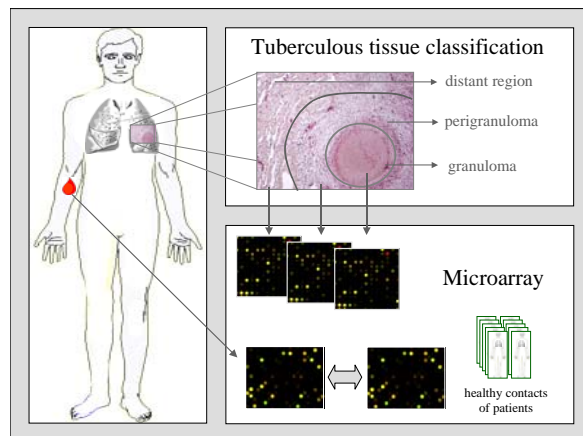
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**Introduction**

Tuberculosis remains a major health threat with 9 million new cases and 2 million deaths annually. The situation becomes worse with increasing incidences of multi-drug resistant (MDR) tuberculosis, amounting to more than 300.000 new MDR cases annually (<http://w3.who.org/tb/publichealth.htm>). The vast majority of the estimated 3 billion individuals infected with the etiologic agent *Mycobacterium tuberculosis*, however, is capable of controlling the pathogen, thus preventing disease outbreak. Only in less than 10 % of all infected individuals active disease develops, mostly through reactivation of dormant microorganisms and less frequently through exogenous re-infection. The mechanisms underlying resistance against tuberculosis remain largely unknown. Even the best-defined marker of protective immunity against tuberculosis, interferon gamma (IFN $\gamma$ ), produced by antigen specific T lymphocytes represents an insufficient correlate of protection. It is most likely that resistance against tuberculosis is polygenic and hence, requires definition of multiple genetic parameters. Here we aim at identifying characteristic immunologic features of human tuberculous lung samples and peripheral blood from patients by global transcriptome analyses.

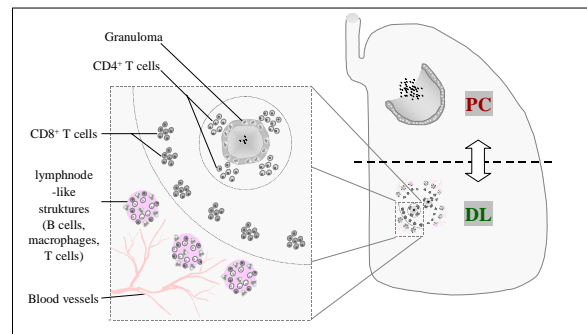
**Transcriptome pattern from tuberculosis diseased pulmonary tissue**

In close collaboration with the Central Tuberculosis Research Institute (CTRI) pulmonary tissue specimen are obtained from patients suffering from MDR tuberculosis disease in Russia undergoing lung resection for medical reasons. Samples are classified according to mycobacterial burden and immune cell distribution. Transcriptome comparisons include differentially affected tissue samples from individual tuberculosis patients, patients suffering from non-infectious insult (lung cancer), and healthy pulmonary tissue. Gene expression profiling is performed using human whole genome oligonucleotide microarrays (Agilent technologies) (Figure 1).



**Fig 1:** Identification of characteristic gene expression pattern in pulmonary tissue and peripheral blood from tuberculosis patients and controls.

Taking advantage of immunohistological analyses of tuberculosis affected pulmonary tissue<sup>1,2</sup> demonstrating that infiltration of immune cell populations and activity were concentrated in well-organized active centers in distant lung tissue we wondered whether the general organization of the granulomatous tissue in human lungs resembles secondary lymphoid organ structures (Figure 2).



**Fig 2:** Highly organized immune cell infiltrates resembling secondary lymphoid-like structures in the lung from tuberculosis patients. PC: pericavitary tissue; DL: distant lung tissue.

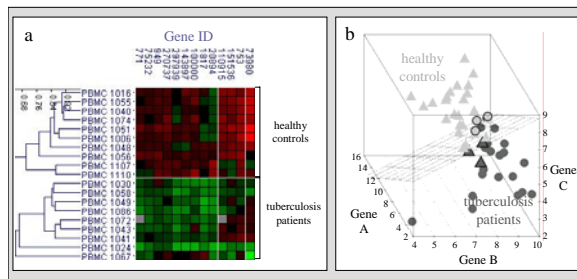
Interestingly, first results from gene expression comparisons between pericavitary and distant lung tissue samples from three tuberculosis patients reveal differences in chemotactic molecules crucial for the recruitment of B cells and follicular T helper cells (e.g. CXCL-13). Additionally, molecules with described functions in granuloma formation namely osteopontin and the monokine induced by IFN-gamma (MIG) were up-regulated in pericavitary tissue. While differences between differentially affected tissue samples from tuberculosis patients were modest, marked changes were found comparing tuberculous and healthy pulmonary samples. Immune cell activation markers, mediators of inflammation, and tissue damage related molecules were highly up-regulated in tuberculous tissue.

**Outlook**

To further analyse the regulation of the local immune response during persistence or active disease, we will isolate regions of interest by laser capture microdissection from lung tissue of both latent and active TB patients, i.e. i) wall structures of well-contained tuberculomas or caseotic cavities; ii) productive granulomas and peripheral leukocyte infiltrations. Comparison with lung cancer specimen will reveal whether gene expression differences are specific for tuberculous pulmonary tissue specimen.

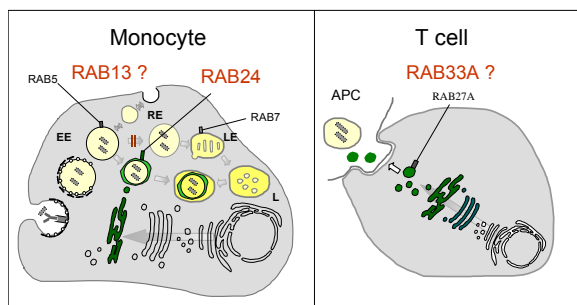
### Gene expression pattern of peripheral blood immune cells from tuberculosis patients and healthy controls

The aim of this approach is to determine tuberculosis disease/infection specific biomarkers in peripheral blood immune cells from tuberculosis patients and healthy contacts. We collected blood samples from 62 tuberculosis patients and 47 healthy contacts. Mononuclear cells were isolated, characterized for the composition of immune cell populations, and RNA was prepared to determine gene expression. Analyses demonstrated the necessity of adjustment for cellular heterogeneity (deconfounding) to improve the validity of transcriptome interpretation<sup>2</sup>. This way we identified differential gene expression pattern capable of discriminating tuberculosis patients from healthy controls (Figure 3a). Linear discriminant analysis revealed a minimal gene cluster optimal for discrimination between tuberculosis patients and healthy controls (Figure 3b).



**Fig 3:** Classification of tuberculosis patients and healthy controls using gene expression pattern. Gene ID: gene identifier.

Furthermore these comparisons revealed differences in the regulation of Ras-associated small GTPases (Rab). Rab molecules are specific regulators of intracellular vesicle trafficking. Since interference with host cell vesicular transport is a hallmark for many intracellular pathogens with the notable example of *M. tuberculosis* we investigated several Rab family members in detail. Rab13, Rab24, and Rab33A were significantly differentially expressed between tuberculosis patients and controls<sup>4</sup> (Figure 4).



**Fig 4:** Possible roles of Rab13, Rab24, and Rab33A in the host immune response against tuberculosis infection. APC: antigen presenting cell; EE: early endosome; RE: recycling endosome; LE: late endosome; L: lysosome.

Rab24 which was preferentially expressed in monocytes was up-regulated in tuberculosis patients. Rab24 may participate in fusion events between the endoplasmic reticulum/cis-Golgi compartment and lysosomes controlling autophagy processes. Very recently an important role of autophagy in the host immune response against *M. tuberculosis* infection has been observed. This study revealed that autophagy abrogates the block of phagosome maturation leading to increased *M. tuberculosis* elimination within macrophages.

Rab13 is preferentially expressed by epithelial cells crucially involved in the regulation of functional tight junction assembly. Recently the role of Rab13 in this process has been further clarified. Morimoto et al. demonstrated that Rab13 specifically regulates continuous endocytic recycling in epithelial cell lines. The specific role of Rab13 in the host immune response is unknown.

Rab33A was down-regulated in tuberculosis patients and predominantly expressed in CD8<sup>+</sup> T cells. Limited information is available about the function of Rab33A. Formerly known as S10, Rab33A was discovered in the early nineties and found to be expressed in the human T cell line Jurkat and the monocyte cell line U937. Rab33A fulfills all criteria of small GTP-binding proteins and, therefore, its role in the regulation of intracellular vesicle transport or signaling is likely. We excluded possible influences of differences in T cell proportions between both study groups demonstrating Rab33A gene expression changes on single cell level. In vitro, Rab33A mRNA expression was induced upon TCR ligation and by dendritic cells (DC) pulsed with *M. tuberculosis*. These findings identify Rab33A as a T cell regulatory molecule in tuberculosis and suggest its involvement in disease processes. Rab33A is an X-chromosomally coded gene located at Xq26, a region found to be associated with tuberculosis susceptibility in an African population. In close collaboration with the project NIE-S17T19 (Dr. Meyer, BNI, Hamburg) we started an initial case-control study to determine a possible association of a novel single nucleotide polymorphism (SNP) with disease. No difference in the distribution of this SNP was detected.

### Outlook

Further characterization of the specific role of Rab candidates in the host immune response against infection. To build up a study cohort of Caucasian tuberculosis patients and healthy contact family members for the analyses of genes in segregation studies

*Lit.: 1. Ulrichs, T., Kosmiadi, G., et al. (2005). Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. J Infect Dis 192(1): 89-97. 2. Ulrichs, T., Kosmiadi, G. et al. (2004). Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. J Pathol 204(2): 217-28. 3. Jacobsen, M., Repsilber, D. et al.; Deconfounding microarray analysis: Flowcytometry resolves tissue heterogeneity bias. Submitted for publication. 4. Jacobsen, M., Repsilber, D. et al., Rab33A, a novel T cell factor, is down-regulated in tuberculosis patients. J Infect Dis, in press.*