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THE INFLUENCE OF THE H2 COMPLEX ON *MYCOBACTERIUM AVIUM* INFECTION IN MICE

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Mycobacterium avium is the opportunistic pathogen in humans, animals and birds and the most common cause of non-tuberculous mycobacterial lung infections worldwide. Analogously to other mycobacterial infections, its antigens are presented predominantly in the context of the Class II *MHC* molecules resulting in activation of $CD4^+$ T cells producing IFNγ, the key cytokine in antimycobacterial response and infection control.

Addressing genetic control of *M. avium*-triggered disease, we compared two congenic strains of mice on the B6 genetic background established in our lab — B6.I-100 ($H2\text{-}A^jE^j$) and B6.I-139 ($H2\text{-}A^{jb}E^j$) — that carry different alleles encoding the β-chain of the *H2-A* gene. After aerosol *M. avium* challenge, B6.I-139 mice died earlier and displayed more severe cachexia compared to B6.I-100 mice. Measurement of the CFU counts in lungs and spleens at weeks 8, 12 and 18 post infection, revealed significant differences in the lung phenotype at the early phase (more CFUs in the lungs of B6.I-100 mice). Assessment of lung pathology demonstrated diffuse inflammation in the lung tissue of B6.I-139 mice at week 8 post infection and granulomata containing foamy macrophages and necrotic zones during the chronic phase. Flow cytometry and immunohistochemical staining revealed higher neutrophil inflammation in the lungs of B6.I-100 mice, accompanied by an increased expression of genes involved in neutrophil attraction. The level of proinflammatory TNFα, but not IL-6 and anti-inflammatory IL-10 and TGF-β, was higher in the lungs of B6.I-100 at an early stage of infection. Importantly, lung $CD4^+$ T cells from more resistant B6.I-100 mice were more activated ($CD44^{hi}CD62L^{lo}$ phenotype) and produced significantly more IFNγ in response to mycobacterial antigens during chronic stage of infection. Higher numbers of lung $CD4^+$ T cells in B6.I-139 mice in 8 weeks after challenge may reflect an attempt of the host to control infection early after challenge, apparently not successful. Of note, it is not clear whether interstrain differences in disease progression reflect differences in the efficacy of antigen presentation between $H2\text{-}A^b$ alleles and subsequent T cell activation, or T cell exhaustion during chronic stage of the immune response. Overall, our data suggest that the allelic differences in the H2-A molecule are involved, albeit moderately, in control to *M. avium* infection.

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MYCOBACTERIUM AVIUM-TRIGGERED DISEASE: HOST GENETICS AND IMMUNITY IN MOUSE MODELS

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Mice of the I/St strain are extremely susceptible to *Mycobacterium tuberculosis* but resistant to *M. avium* infection, whereas B6 mice show a reversed pattern of susceptibility. By directly comparing: (i) characteristics of susceptibility to two infections *in vivo* (ii) architecture of lung granulomata and (iii) expression of genes encoding regulatory factors of neutrophil influx in the lung tissue, we demonstrate that genetic susceptibility of the host determines the pattern of lung pathology. *M. avium*-infected B6 mice and *M. tuberculosis*-infected I/St mice are prone to develop necrotizing granuloma surrounded by hypoxic zones, massive neutrophil influx and B-cell follicles in the lung tissue. These mirror-type lung tissue responses demonstrate that the level of genetic susceptibility of the host to a given mycobacterial species largely determines characteristics of pathology, and emphasize the importance of host genetics in pathogenesis. Segregation genetic analysis and development of novel H2-recombinant congenic strains allowed dissection of genetic control of two infections. Regarding susceptibility to and severity of *M. avium*-triggered disease, involvement of two distinct genes was clearly demonstrated: the *Slc11a1* (former *Nramp1*) gene, acting as a major genetic factor, and the classic Class II *MHC* gene *H2-Ab*, a minor modifier of susceptibility pattern.

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EVOLUTION AND TRANSMISSION OF *MYCOBACTERIUM TUBERCULOSIS* RESISTANCE TO FLUOROQUINOLONES

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Fluoroquinolones (FQs) have been widely used for the tuberculosis (TB) treatment for decades and *Mycobacterium tuberculosis* strains resistant to FQs have been reported globally. In the past few years, we had gained some insights into the evolution and transmission of *M. tuberculosis* FQ-resistance. Firstly, we found that FQ-resistance mostly appeared in multi-drug resistant (MDR) *M. tuberculosis* strains. We observed the emergence and transmission of FQ-resistance in clinical clustered (as defined by whole-genome sequencing) MDR cases and we speculated that the general inclusion of FQs in the first-line treatment regimen in western China may contribute to the high resistance rate among MDR cases. By studying the within-host heterogeneity of *M. tuberculosis*, we proved that the evolution of FQ-resistance is associated with the emergence and competition of several resistance related mutations in DNA gyrase genes, a process for selecting highly resistant and low-cost strains. Lastly, we studied the mechanisms of primary ofloxacin-resistant strains to acquire resistance to moxifloxacin (new generation FQ) and we found a secondary mutation in DNA gyrase associated with this process.

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EPIDEMIOLOGY OF EXTRAPULMONARY TUBERCULOSIS IN ALBANIA, 2010–2016

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Extrapulmonary tuberculosis (EPTB) is a therapeutic challenge. Possible reasons include ist under- and overdiagnosis/reporting. Here, we report results of the cross-sectional retrospective review of the epidemiology of EPTB in Albania from 2010 to 2016.

The objectives of the study were to find out epidemiological characteristics of EPTB and to explore risk factors, and challenges in the diagnosis and management of EPTB in Albania.

We used data from National TB Program and included all cases of TB diagnosed in the Albania from 2010 to 2016. Information on age, sex, year of diagnosis, and anatomic location of the site of disease was retrieved from central database of National TB Program.

In Albania during 2010–2016, 925 cases of extrapulmonary TB were reported, males were 581 (63%) and females 344 (37%). The number of cases diagnosed per year was as follows: 170 (38.2%) in 2010, 129 (30%) in 2011, 108 (25.7%) in 2012, 141 (29.7%) in 2013, 147 (36%) in 2014, 117(28.2%) in 2015 and 113 (27.2%) in 2016.

Sputum smear examination, X-ray and culture examination and tissue biopsy were carried out in 58; 42.3; 18 and 15% of patients respectively for EPTB diagnosis. The most affected age group was < 65 years (23%). Pleural effusion (35%) and lymph node (15.7%) were the most common types of extrapulmonary TB.

Patients live in urban areas (60%) rather than rural (40%). The mean age of EPTB patients is 44.5 and pulmonary TB patients is 41.2. Incidence of EPTB decreased from 5.5/100 000 in 2010 to 5.1/100 000 in 2016.

In Albania, extrapulmonary TB in 2010–2016 showed a slight decrease in incidence, although the rates are still very high. Diagnosis of extrapulmonary TB was made according to national guidelines, however long delay has been reported in most cases before the final diagnosis. Microbiological proof is the key to diagnosis and treatment, and tissue biopsy that should be required regularly.

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DETERMINANTS OF TB RELATED DEATH FROM TUBERCULOSIS PATIENTS IN THE NORTHERN THAILAND

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Tuberculosis is the most common cause of deaths from respiratory infection in Thailand. Understanding the risk of death could provide useful information to provide better clinical care for TB patients. *N-Acetyltransferase 2 (NAT2)* gene is the main determinant of isoniazid (INH) metabolism. *NAT2* rapid acetylator contributes to lower anti-TB drug (INH) serum concentration and increased risk of treatment failure and relapse from INH based TB regimens.

The aim of this study was to determine the effect of *NAT2* acetylator status on TB related death.

TB patients were recruited from the TB registry during 2002–2011 in Chiang Rai province, Thailand. The *NAT2* acetylators (rapid, intermediate and slow) were determined by haplotype specific polymerase chain reactions, HS-PCR. These groups of patients were excluded from further analysis: 1) patients who did not receive the INH based regimens for TB treatment; 2) patients who did not receive the INH based regimens longer than 2 weeks and 3) patients who died within the first 2 weeks of TB treatment. Mortality-associated risk factors within 1 year of treatment were analyzed using Cox-regression model.

Of 1.076 TB patients who met study criteria, 213 (19.8%), 495 (46.0%) and 368 (34.2%) belonged to *NAT2* rapid, intermediate and slow acetylate group respectively. In total, 115 patients died within 1-year follow-up. In the multivariate analysis, rapid *NAT2* acetylator status increased the risk of death when compared against *NAT2* intermediate acetylator (adjusted hazard ratio [aHR]: 1.83, 95%CI: 1.15–2.91). The effect of *NAT2* rapid acetylator on deaths is more significant in HIV positive TB patients (aHR 2.68, 95%CI:1.14–6.26). The risk factors associated with death was different among the *NAT2* acetylators. In *NAT2* rapid acetylator group, elderly people, HIV positive, past TB history and smoking status was increased the risk of death.

The *NAT2* rapid acetylator is related to the mortality during TB treatment. The inadequate treatment with the doses of standard regimens caused by *NAT2* rapid acetylator might increase the risk of death in TB patients, these NAT2 pharmacogenetic risks are interacting with other clinical risk factors, which is depended on the acetylator status.

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CLICHES AND DOGMAS IN MOLECULAR TUBERCULOSIS RESEARCH

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I will present a personal critical view on some hot issues of the molecular epidemiology of tuberculosis, in particular, regarding an uncritical use of some well known online tools and resources. Invaluable for establishing terminology and classification in molecular epidemiological studies of *Mycobacterium tuberculosis*, they are limited by our insufficient knowledge of genome evolution and uncritical perception of their indications. This is exemplified by partly inadequate (sub)clade assignment due to imperfect decision rules, and misleading methodological approach when scientifically unsound phylogenies are built from spoligotyping data.

To begin with, I propose the following definitions. First, "molecular mythology" that relies on minimal array of references that conveniently support long-lasting clichés. Second, "molecular iconography" that relies on dogmatic perception of the current knowledge when online databases are uncritically regarded as ideal icons. Finally, I introduce the term "click science". In contrast to the fascinating and sophisticated click languages, "click science" relies on uncritical and simplified perception of knowledge and a dogmatic, iconographic view of indications provided by increasingly convenient online tools and databases. For example, spolTools is an example of the click phylogenetics when a plethora of statistics is generated in few clicks but their exploration is minimal. In its turn, click systematics is exemplified by SITVIT's (i) reader-unfriendly huge tables with different possible percentages and (ii) easy to read but partly inadequate (sub)clade labels.

Labels are convenient for classification, but should be revisited in the context of modern knowledge. The "we have been taught this way" approach reflects the mentality of a conservative teacher rather than a creative researcher. As Heidegger once said, "knowledge does not think"; indeed why think when it already knows? As far as science is concerned, this quotation from Henry Gee's "The accidental species" is much more appropriate: "Science is about neither Facts nor Truth, but the quantification of doubt".

Below are examples of some clichés pertaining in molecular epidemiology of tuberculosis.

Firstly, pathogenic properties of the Beijing genotype are traditionally listed as increased virulence, association