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MECHANISMS OF PATHOGENESIS

The influence of influenza virus infections on the development of tuberculosis

Roelof A. de Paus [a], Reinout van Crevel [b], Ruud van Beek [c], Edhyana Sahiratmadja [d], Bachti Alisjahbana [e], Sangkot Marzuki [f], Guus F. Rimmelzwaan [c], Jaap T. van Dissel [a], Tom H.M. Ottenhoff [a], Esther van de Vosse [a,*]

[a] Department of Infectious Diseases, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands
[b] Department of Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[c] Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands
[d] Health Research Unit, Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia
[e] Division of Tropical and Infectious Diseases, Internal Medicine Faculty of Medicine, University of Padjadjaran, Bandung, Indonesia
[f] Eijkman Institute for Molecular Biology, Jakarta, Indonesia

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SUMMARY

Recently, it was shown that interferon-γ mediated immune responses, which play a major role in the control of infection with *Mycobacterium tuberculosis* (Mtb), can be inhibited by type I interferons. Since type I interferons are abundantly induced during viral infections, we hypothesized that infections with influenza viruses might play a role in the development of active TB disease either directly after exposure to Mtb or through reactivation of latent Mtb infection. To explore this hypothesis we investigated in a retrospective study whether newly diagnosed adult tuberculosis patients from Indonesia had had recent influenza infection. Plasma samples from TB patients and controls were assayed for antibodies against two subtypes of at that time relevant, seasonal influenza A viruses. Overall, no correlation was observed with the presence of antibodies and manifest tuberculosis. Still, antibody titers against circulating A/H3N2 influenza virus were slightly enhanced in tuberculosis patients as compared to controls, and highest in cases of advanced tuberculosis. This suggests that tuberculosis patients were recently infected with influenza, before clinical manifestation of the disease. Alternatively, the production of antibodies and susceptibility to tuberculosis may be influenced by a common confounding factor, for example the ability of patients to induce interferon-α. We conclude that in an endemic country like Indonesia, an influenza virus infection is not a major determinant for developing clinically manifest tuberculosis.

1. Introduction

Tuberculosis (TB) is a severe disease caused by *Mycobacterium tuberculosis* (Mtb). Up to 10% of the Mtb infected individuals develop active tuberculosis, while the majority of those infected develop a latent state of infection for years.[1,2] During latency, Mtb may stay quiescent, but when the immune system fails to control the bacteria, reactivation may occur and precipitate development of active disease,[3,4] in most cases pulmonary TB disease.

Several risk factors that influence the susceptibility to TB have been described, such as host genetic factors,[5] malnutrition,[6] smoking,[7] diabetes[8] and infection with HIV.[9] Besides these factors also the Mtb strain virulence may influence the course of TB, since virulent Mtb can inhibit the host immune system in various ways.[10] Recently, IFN-α has been described as a putative factor, which may be induced by highly virulent Mtb strains[11,12] and can inhibit an effective IFN-γ mediated immune response.[13–15] Mouse studies revealed that during Mtb infection IFN-α is induced and that IFN-γ mediated immune responses can be impaired by IFN-α.[12,16] In humans, a typical IFN-α/β transcript signature was found in the blood cells from TB patients.[11,17,18] However, it is not clear whether Mtb infection in humans leads to the production of type I interferons or whether production of type I interferons leads to TB. It is possible that in TB patients type I interferons are more abundantly induced due to infections with viruses, such as pneumotropic influenza viruses.

After influenza virus infection a period of enhanced susceptibility to bacterial infections is commonly seen in humans.[19] In mice, Toll like receptor-induced responses of alveolar macrophages to bacterial ligands remain desensitized for months after an influenza virus infection.[20] This explains why mice are highly susceptible to bacterial pneumonia for several weeks after influenza virus

* Corresponding author. Tel.: +31 71 526 1782; fax: +31 71 526 6758.
E-mail address: E.van_de_Vosse@lumc.nl (E. van de Vosse).

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infection.[21] In humans, post-influenza bacterial pneumonia is a major cause of morbidity,[19] with *Streptococcus pneumonia* as the main pathogen associated with post-influenza pneumonia.[22] TB is usually not diagnosed shortly after influenza infections and although some anecdotal reports suggest that the occurrence of TB was also high during influenza pandemics,[23–25] a causal relationship between the epidemics of the two infectious diseases has not been investigated.

If indeed an influenza virus infection leads to the (re)-activation of a latent Mtb infection, this may remain unnoticed, because the period between (re)-activation of the bacteria and the first presentation of the clinical symptoms of TB is long. TB develops slowly, due to the slow metabolism and replication rate of Mtb. Thus it may be that within the latency period of TB, during the primary infection or after re-infection, a transient inhibitory effect on the antibacterial responses by an influenza virus infection influences the course of TB and leads to active disease, possibly in conjunction with other risk factors. In mice, co-infections of influenza viruses with Mtb enhanced the development of TB in the lungs.[26] In humans, it is still unclear whether influenza virus infections can influence the course of TB.

We hypothesized that influenza virus infections may promote the development of active disease after exposure to Mtb, or might play a role in the reactivation of latent Mtb infection. To explore this hypothesis we investigated in a retrospective study whether patients with clinically-manifest TB had an influenza virus infection recently. Plasma samples collected from TB patients at time of diagnosis and from controls were screened for the presence of antibodies against influenza viruses in order to investigate a putative association between TB and influenza virus infections.

2. Patients, materials and methods

2.1. Study subjects

Patients and controls (Table 1) were recruited between March 2001 and December 2004 from the TB clinic "Perkumpulan Pemberantasan Tuberkulosis Indonesia" in Jakarta.[8,27,28] Patients newly diagnosed with active pulmonary TB, between the age of 15 and 70, were included. TB diagnosis was based on WHO definitions including the presence of clinical symptoms, a chest X-ray examination (CXR), microscopic detection of acid-fast bacilli in sputum and a positive culture of Mtb. Based on the CXR examinations TB patients were classified into two groups; patients with mild to moderate TB and patients with advanced TB. Patients seropositive for HIV were excluded. Community control subjects were recruited from neighboring houses and matched for age, sex and socio-economic class. Controls with a history of TB or with positive TB finding in the CXR were excluded. The control subjects underwent the same examinations as the patients, but were not tested for HIV, since the prevalence of HIV in the Indonesian population was low, as evidenced by the low prevalence of HIV amongst the TB patients in this cohort (1.8%).[29] The influenza vaccination status of our study cohort is unknown. However, at the time of our study, vaccination against influenza viruses in Indonesia was only rarely applied and based on the low socio-economic status of our patients and matched controls these individuals are extremely unlikely to have received such vaccinations. For this study, the patients and controls were matched for the date of inclusion. Patients and matched controls were only included if the dates of inclusion were not more than 14 days apart. Written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia.

Table 1
Description of the study population.

	TB patients ($n = 111$)	Controls ($n = 111$)	p-Value
Age in years (median)	18–67[31]	17–69[35]	0.153*
Gender; males	72 (65%)	60 (54%)	0.132†
Diabetes mellitus	24 (22%)	8 (8%)	<0.001†
Individuals with BCG scar	39 (35%)	45 (41%)	0.489†

* Student t-test.
† χ^2 test.

2.2. Detection of antibodies against influenza viruses

Heparinized plasma samples were obtained from the patients and controls and stored at −80 °C. Thawed plasmas were analyzed for the presence of total IgG and IgM antibodies against two subtypes of influenza A virus, a H3N2 virus (A/Moscow/10/99, vaccine strain ResVir-17) and a H1N1 virus (A/New Caledonia/20/99, vaccine strain IVR-116), using the hemagglutinating inhibition (HI) test. These strains were chosen because of their high antigenic similarity to the specific H3N2 and H1N1 viruses circulating during the sample period. For use in the HI test, the influenza virus strains were propagated in 11-day old embryonated chicken eggs. The HI test was performed in duplicate according to standard methods[30] with turkey erythrocytes and four hemagglutinating units of virus. Ferret sera raised against the test antigens were used as positive controls. All plasma samples of all study subjects were tested simultaneously, in duplicate, and were only regarded positive when both analyses gave positive results. The threshold of detection was an HI titer $\geq$10.

2.3. Statistical analysis

Data were analyzed using SPSS software. The Pearson χ^2 test and the paired samples t-test were regarded significant when $p < 0.05$.

3. Results

3.1. No correlation between the incidence of tuberculosis and the seroprevalence of antibodies against influenza viruses

To study the effect of influenza virus infections on TB we examined the plasma samples of controls and newly identified TB patients for the presence or absence of antibodies against two subtypes of influenza A virus, H1N1 and H3N2 (Table 2). The threshold of detection was an HI titer $\geq$10. Both influenza strains circulated in the population during the time of plasma sampling: 46% of the TB patients and 41% of the controls had antibodies against H1N1, while 82% of the TB patients and 82% of the controls had antibodies against H3N2 (Table 2). No significant differences in the number of individuals with antibodies against influenza viruses were thus observed between the control group and the group of TB cases.

Table 2
Seroprevalence of antibodies to A/H1N1 and A/H3N2 influenza viruses.

Group	H1N1 positive	H3N2 positive
TB patients ($n = 111$)	51 (46%)	91 (82%)
Controls ($n = 111$)	46 (41%)	91 (82%)
Total ($n = 222$)	97 (44%)	182 (82%)
χ^2 test; p-value	$p = 0.499$	$p = 1.000$

The threshold of detection was an HI titer of $\geq$10.