Viejos y nuevos enfoques para el desarrollo de vacunas contra la tuberculosis

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¿Necesitamos esfuerzos renovados para desarrollar nuevas vacunas para combatir la tuberculosis? Los datos epidemiológicos muestran que la disminución de la ya baja incidencia de tuberculosis se ha detenido en los países en desarrollo. En ciertos países occidentales la incidencia de tuberculosis ha aumentado incluso en los últimos 10 años. En Africa y Asia se encontró una alta incidencia de tuberculosis similar a la del mundo occidental en los años 30. ¿Podemos predecir por la historia de la tuberculosis en Europa Occidental que la epidemia de tuberculosis en los países en desarrollo ya alcanzó su pico o se encuentra todavía en aumento? Datos revisados muestran que en Europa la epidemia de tuberculosis comenzó al menos tres siglos antes de alcanzar su apogeo a mediados del siglo XIX. Las razones para la disminución de la tuberculosis en la primera mitad del siglo XX en el mundo occidental todavía no son bien comprendidas. ¿Las medidas de salud pública o el adecuado tratamiento con antibióticos reducirán o detendrán la tuberculosis en Africa y Asia o continuará aumentando la tuberculosis? Nuestra posibilidad para controlar la diseminación de la enfermedad se complica por la aparición de cepas resistentes a antibióticos y el VIH. Por lo tanto se requiere una mejor comprensión de la base molecular de la interacción entre el bacilo y sus huéspedes para el desarrollo de mejores enfoques de tratamiento e inmunización. La inmunidad celular y la hipersensibilidad de tipo retardado (HTR) son procesos claves en el curso de la infección por el *Mycobacterium* y están involucradas tanto en la infección primaria como en la secundaria, así como en la inducción de la inmunidad protectora en el huésped. Los diferentes tipos de vacuna contra la tuberculosis que están siendo reevaluadas incluyen: BCG con dosis de refuerzo, BCG oral, BCG modificada (multivalente), cepas auxotróficas atenuadas del *M. tuberculosis*, proteínas secretadas o recombinantes de *M. tuberculosis* con o sin inmunomoduladores y vacunas de ADN. Estas nuevas vacunas inducen una buena inmunidad celular y pueden contribuir al desarrollo de enfoques mejorados de inmunización y tratamiento.

Palabras claves: Vacunas, tuberculosis, *Mycobacterium tuberculosis*

A rapid history of tuberculosis

M. tuberculosis evolved from *M. bovis* among milk-drinking Indo European who then spread the disease during their invasion or migration into Western Europe and Eurasia. This statement based on a hypothetical phylogeny of *M. bovis* and *M. tuberculosis*, on anthropology, and on correlation between lactose malabsorbers and incidence of tuberculosis morbidity and macrophage permissivity to *M. tuberculosis* has never been proved with hard data (6, 9, 16). The finding of *M. tuberculosis* in prehistoric American bison (H. Bercovier, unpublished data) and in pre-Columbian mummies in the Americas negate this theory.

It is accepted that tuberculosis culminated in the 19th centuries in Europe with a continuous decrease in the 20th century even before vaccination or antibiotic treatments were available (6, 9). At the end of the 19th century and at the beginning of the 20th century, the high prevalence of tuberculosis began to decrease. As an example, the death rate due to tuberculosis in New York was 750/100,000 in 1805 and decreased to 400/100,000 in 1870 (9). Such a dramatic decrease has not yet found a solid explanation. We hypothesize that the epidemic of tuberculosis in the Western world started at the end of the middle age. A correlate of our hypothesis is that the social changes that happened in the late Middle Age were a major factor in the epidemization of tuberculosis. Society in Europe changed from an agricultural scattered fixed society to a mobile bourgeois society. Non exposed European populations that remained rural such as in Ireland remained susceptible to tuberculosis even in the 20th century.

In the 17th century, the registry in England and Wales in 1650 show that pulmonary phthisis accounted for 20 % of all deaths, mainly in cities. The first decree of prophylaxis against tuberculosis dates from 1699, in Italy (9). These facts allow us to suppose that tuberculosis was already a serious health problem during the 17th century. Pathological findings and ancient DNA analysis in a late medieval population of Lithuania indicates that between 25 to 50% of the population was infected with *M. tuberculosis* making a 10-20% death rate credible (10). In Europe, leprosy was epidemic in the early Middle Age and stopped its progress to decline significantly during the Renaissance. A simplistic theory to explain the decrease of leprosy was the improvement of hygiene and the general cooling of the weather. Sporadic cases of leprosy occurred in Europe until the 20th century but the last European indigenous case of leprosy occurred in the clean and cold Norway in 1953 rendering these theories doubtful. The exclusion of leprosy by tuberculosis, an hypothesis proposed by Chaussinaud 50 years ago (3), is now supported by experimental data in mice (live BCG or BCG extracts protect against *M. leprae* infection) (19) and by studies in humans in

Africa (14), in Asia (2) and in South America (15) that showed the protective role of BCG against leprosy.

What social change may have help tuberculosis to become epidemic in the 15th century? The changes from a completely scattered population depending on the local feudal power to a more structured society with a centralized power favoring small cities have probably implanted tuberculosis. The development of commerce has, at the same time, favored the large diffusion of the disease.

Do we need vaccines for tuberculosis?

Based on case notifications and on estimation, the World Health Organization (WHO) estimates that 88 million new cases of tuberculosis occurred or will occur during the decade 1990-1999. Among those 88 million new cases of tuberculosis 30 million died or will die (5, 8, 21, 23). The vast majority of the cases of tuberculosis are nowadays occurring among inhabitants of Asia and Africa whereas less than two per cent of all the new cases concern residents from industrialized countries.

In certain Western countries the incidence of tuberculosis has increased in the last ten years but the rates of the incidence are so low that the risk of a new epidemic seems impossible at the moment (4, 8). HIV and MDR strains of tuberculosis are the main factors for the actual relative increase of tuberculosis in industrialized countries. A large segment of the population in industrialized countries has still herd immunity due to exposure to *M. tuberculosis* in the 40s. However, within 20 years most of the exposed population will disappear and as a result we could see new foci of epidemics in the Western world if the public health institutions are not prepared to detect rapidly the disease. Such a situation has been recently seen with an epidemic of diphtheria in Eastern Europe. Published data show clearly that tuberculosis is reemerging in Eastern Europe (4).

To analyze the current situation and to predict the future, we must try to figure out if the major changes that could be related to the development of the epidemic of tuberculosis in Europe, as analyzed before, are met now in Africa and Asia. From this analysis, we could predict if the epidemics of tuberculosis in Africa and Asia have already reached its apogee or if it is in development. Are Asia and Africa following a small urbanization, from agriculture to small cities like in Europe in the late Middle Age?

The Asian tuberculosis epidemic could mimic that of Europe and could result in the future in an increasing incidence of tuberculosis (8, 13). Will selection occur in Africa and Asia as it may have occurred in Europe and will it take four centuries to produce a more resistant population? Will HIV and *M. tuberculosis* MDR strains entangle the epidemiological evolution of tuberculosis? Anyhow the best option is to be ready to control tuberculosis better than we do it today. An effective vaccine, which would also prevent the stage of carrier, could resolve all the problems due to tuberculosis.

Old and new approaches for vaccines against tuberculosis

The only vaccine available against *M. tuberculosis* is BCG, a live attenuated vaccine (22). BCG (Bacille Calmette-Guerin), an attenuated strain of *M. bovis* has been used worldwide as a human vaccine against tuberculosis. Although, the use of BCG has been successful in England or in the USA, it has been less effective in other countries (22). The protective efficacy of the BCG vaccine varied in different trials from 0 to 80%. BCG administrated at birth clearly protects children against tuberculous meningitis. New approaches for the use of BCG comprise the revaccination of young children at 10-15 years of age. Oral administration of BCG with a protecting carrier could prevent side effects of BCG revaccination (G. Marchal, personal communication). Recombinant BCG with multivalent antigens could be even more cost efficient in developing countries. New auxotrophic strains of M. tuberculosis are potential candidate to improve vaccination efficacy, but without any supporting experimental data However, in immuno-compromised patients, these live attenuated strains cannot be used. Cellular immunity and delayed type hypersensitivity (DTH) are key processes in the course of mycobacterial infection and are involved in both primary and secondary infection as well as in the induction of protective immunity in the host (1, 20). Only acellular vaccines can provide both safety and immunogenicity to protect immuno-compromised and normal patients. Data on acellular vaccines against tuberculosis are meager and based on excreted proteins (12). Secreted proteins of *M. tuberculosis* administrated in guinea pigs with an adjuvant can give a protection of two logs whereas BCG gives a three-log protection. Addition of lymphokines to these proteins gives three logs protection in mice challenged with a virulent *M. bovis* strain. Acellular vaccines against intracellular multiplying bacteria are yet to be shown superior to BCG. The same can be said about recombinant proteins where one to three logs protection can be attained when adding immunomodulators such as interferon gamma (11, Bercovier H. unpublished data). Naked DNA has been used of for vaccination against viral diseases (influenza, hepatitis B, HIV, rabies, hepatitis C, and herpes simplex), parasitic diseases (malaria, leishmaniasis, schistosomiasis), and bacterial diseases (7, 17, 18, 24, 25). The results are encouraging, demonstrating distinct advantages of DNA vaccines over conventional vaccines: processing through the MHC I and MHC II pathways; induction of humoral and CTL responses increased by the presence of CpG sequences; requirement for small amounts of DNA (µg range) compared with the mg level needed for proteins and peptides; a long protective immunity after a single inoculation; easy preparation and preservation of plasmid DNA which may eliminate the need for the "cold chain" required for conventional vaccines. The DNA is not immunogenic by itself and it remains episomal. However, in

spite of the great potential of DNA vaccination, it still faces safety uncertainties. So far, only the i.m. injection of naked DNA into the tibialis anterior muscle has proven effective and reproducible in mice.

Recently, promising results were reported by vaccinating mice with a plasmid DNA encoding the *M. tuberculosis* 85 antigen complex which resulted in a Th1 helper cells T-cell response (18, 24). The level of protection obtained by these DNA vaccines can be measured by a decrease of the bacterial load in certain organs (spleen, lung) of 1 or maximum 2 log of the bacterium used for challenge. These results are equal or inferior to the level of protection obtained when using BCG. Similar results were obtained by Lowrie et al. (17) and by us (Bercovier, unpublished data) using plasmid DNA encoding for

additional *M. tuberculosis* genes (hsp65, hsp 70, 36 kDa, 6 kDa, rplL). Moreover, these vaccines induce a good cellular immunity (increase in both antigen specific $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells and presence of CTL) concomitant with the production of IFN, IL-2, IL-12 and TNF . Although the DNA vaccines did not prove yet that they were superior in their protective activity to the BCG vaccine, we suggest that in accordance to their immunogenic properties they should be used as therapeutic vaccines.

In order to control efficiently the spread of tuberculosis, the scientific community will have to face the challenge of developing a therapeutic vaccine against tuberculosis able to stop the transmission of the disease.

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Old and New approaches for Vaccines against tuberculosis

Abstract

Do we need renewed efforts to develop new vaccines to fight tuberculosis? Epidemiological data show that the decrease of the already low incidence of tuberculosis has stopped in developed countries. In certain Western countries the incidence of tuberculosis has even increased in the last ten years. In Africa and in Asia, a high incidence of tuberculosis is found similar to that of the Western world in the 1930s. Can we predict from the history of tuberculosis in Western Europe that the epidemic of tuberculosis in developing countries has reached his peak or is still developing? Revisited data from Europe show that the epidemic of tuberculosis started at least three centuries before it reached its apogee in the middle of the 19th century. The reasons for the decrease of tuberculosis in the first half of the 20th century in the Western world are still not well understood. Will public health measures and proper antibiotic treatment reduce and stop tuberculosis in Africa and in Asia or will the incidence of tuberculosis increase? Our ability to control the spread of the disease is complicated by the appearance of antibiotic resistant strains and HIV. Therefore, a better understanding of the molecular basis for the interaction between the bacilli and the hosts is necessary for the development of improved approaches for treatment and immunization. Cellular immunity and delayed type hypersensitivity (DTH) are key processes in the course of mycobacterial infection and are involved in both primary and secondary infection as well as in the induction of protective immunity in the host. The different types of tuberculosis vaccines being reevaluated comprise: BCG with booster, oral BCG, modified BCG (multivalent), auxotrophic *M. tuberculosis* attenuated strains, *M. tuberculosis* secreted proteins or recombinant proteins with or without immunomodulators and DNA vaccines. These new vaccines inducing a good cellular immunity may contribute to the development of improved approaches for immunization and treatment.

Key words: Vaccines, tuberculosis, *Mycobacterium tuberculosis*