Rev. salud pública. 12 sup (2): 26-30, 2010

Immune response

Clinical implications of basic research in tuberculosis

Carlos A. Parra-López

Department of Microbiology Universidad Nacional de Colombia-Medical School. Bogota, Colombia. South America.

Although one in every two individuals in the world have been vaccinated against tuberculosis with the BCG vaccine, currently is estimated that one third of the world population is infected with Mycobacterium tuberculosis (*M. tuberculosis*) the bacillus that causes tuberculosis. Each year, *M. tuberculosis* is responsible of 8 million new cases of tuberculosis and kills 2.5 million individuals in countries worldwide. These ciphers, together with the increase of cases of tuberculosis caused by strains multi-drug-resistant (MDR) and extremely resistant (XDR) to treatment with chemotherapeutic agents, makes basic research aimed to elucidate basic mechanisms of pathogenicity, drug resistance and evasion of the immune response of the bacillus an urgent need. As introduction to the basic research symposia of the congress, in this presentation I will highlight recent achievements on molecular genetics, bacterial pathogenesis and immunology of tuberculosis, performed by several outstanding scientists who kindly accepted the invitation to our congress. As we will see, their work have made possible not only the identification of new virulence factors of the bacillus but also the discovery of new mysteries of the biology of infection of *M. tuberculosis* into macrophages and a better understanding of the human immune response against the bacilli. Altogether their research has blossomed in important advances in diagnosis and identification of new biological markers of the immune response against tuberculosis. We hope that altogether these great achievements of basic research will contribute in the near future to the design of better diagnostic and more potent immuno-prophylaxis tools against *M. tuberculosis.*

The mysteries of making antigens presentable to the immune system

Emil R. Unanue

Division of Immunology, Department of Pathology and Immunology, Washington University, St Louis, MO, USA, 66110.

Selection of peptides from the processing of proteins leading to their interaction with histocompatibility (MHC) molecules is the first and central step in antigen presentation, the processs that leads to recruitment and activation of T lymphocytes. Peptides are selected and bound to class II-MHC molecules, depending on key sequence motifs that vary depending on MHC alleles. The peptide-MHC complex which is displayed on Antigen Presenting cells (APC) can now be identifed, examined biochemically, and quantitated on APC, both ex vivo and in vivo.This examination gives new perspectives on the nature of the ensuing CD4 T cell response. Among the selected peptides, a number have suffered post-translational modifications that generate highly unique T cells. For one example after infection, some T cells recognize nitrated tyrosines or oxidized tryptophans in residues from microbial peptides. Other T cells recognize very specifically a spectrum of different

conformational isomers of a peptide-MHC complex. While chemistry of peptide selection by, and interaction with, class II-MHC molecules is reasonably well understood, the other side of the equation, that of the T cell response is still to be developed. This issue is of particular importance in the context of vaccinations. Variables that influence T cell selections include: the dissociation rate of peptides bound to MHC molecules, the density of pMHC on the APC, competition and cooperativity among T cell clones, the intrinsic adjuvanticity of the antigen, the T cell repertoire developed in the thymus, and the regulatory interactions that expand or limit the T cell repertoire.

A germline but macrophage-tropic CYBB mutation in patients with X-linked predisposition to mycobacterial diseases

Jacinta Bustamante[1,2], Guillaume Vogt, Lizbeth Blancas-Galicia[1,2], Carolina Prando[1,2], Stephanie Boisson-Dupuis[1,2] Peter Newburger[3], Capucine Picard[1,2,4], AntonioCondino-Neto [5,6], Abel Laurent[1,2], Jean Laurent Casanova[1,2,7]

1 University Paris René Descartes,Necker Medical School, 75015, Paris, France, EU.
2 Laboratory of Human Genetics of Infectious Diseases, INSERM U550, 75015 Paris, France, EU.
3 University of Massachusetts Medical School, Worcester, USA.
4 Center for the Study of Primary Immunodeficiences, Necker Hospital, 75015 Paris, France, EU.
5 Department of Pediatrics and Pharmacology, State of Campinas Medical School, 13081-970, Campinas SP, Brazil.
6 Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, 05508-900 Sao Paulo SP, Brazil.
7 Pediatric Hematology-Immunology Unit, Necker Hospital, 75015, Paris, France, EU.

Germline mutations in human X-linked CYBB, encoding the gp91 subunit of the NADPH oxidase, impair the respiratory burst of all types of phagocytes. The corresponding condition, chronic granulomatous disease (CGD), is a pediatric illness associated with widespread granulomas and multiple fungal and bacterial infections, including mycobacterial diseases. We report here a multiplex kindred in which the otherwise healthy adult males show X-linked recessive Mendelian susceptibility to mycobacterial diseases (MSMD; BCG disease and tuberculosis). The patients harbor a new mutation in CYBB that abolishes the respiratory burst in monocyte-derived macrophages but not in monocytes or granulocytes. The macrophage-specific functional consequences of the germline mutation result from the cell-specific impairment of NADPH oxidase assembly. Macrophage-tropic CYBB allele-associated MSMD therefore recapitulates and explains the mycobacterial phenotype of patients with CGD. This experiment of nature indicates that the respiratory burst in human macrophages is a crucial effector mechanism for protective immunity to mycobacteria — *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* in particular. Conversely, granulomas and other infections affecting patients with CGD are consequences of additional granulocyte and/or monocyte dysfunction.

Role of CD1 antigen presentation to t cells and NKT cells in *M. tuberculosis* infection

Michael B. Brenner

Brigham and Women's Hospital and Harvard Medical School, Boston, MA. USA.

CD1a, b, c and d molecules present an array of lipid antigens from the M. tuberculosis including mycolic acids, mycolates, sulfolipids, polyketides, phospholipids, and lipopeptides. CD1 molecules resemble MHC I except that they have hydrophobic channels that bind acyl chains rather than peptides. CD1a, b and c elicit adaptive T cell responses in humans and expand lipid antigen specific T cells during and after infection. In contrast, CD1d bearing APC elicit innate-like NKT cells rapidly following infection. These T cell subsets are Th1 cytokine produces and CTL that not only kill infected APC but also are bactericidal for mycobacteria. The nature of the antigens and how CD1 molecules survey intracellular endosomal compartments to bind and present them will be outlined as will key differences between CD1 and MHC antigen presentation. The potential role of CD1 antigen presentation in vaccine development will be considered.

T cells and tuberculosis: what are they doing?

JoAnne L. Flynn

Department of Microbiology and Molecular Genetics and the Center for Vaccine Research. University of Pittsburgh School of Medicine. Pittsburgh PA United States.

CD4 and CD8 T cells are induced in response to *M. tuberculosis* infection. The specific or protective roles and functions of these cells at different stages of infection are not clear. Using two different animal models, we have studied the requirement for each type of T cell as well as the function of these cells in the immune response to *M. tuberculosis*. We have found that CD8 T cells are necessary for control of initial infection, as well as chronic and possibly latent infection. These cells produce cytokines, albeit less IFN-g than CD4 T cells in response to M. tuberculosis infection. CD8 T cells can also be cytotoxic but, unlike in other infections, IFN-g production and cytotoxic activity are not expressed by the same individual cell. Using an adoptive transfer system, we demonstrated that IFN-g production by CD4 T cells is essential to control of infection, even if other cells can produce this cytokine. We also demonstrate, in a non-human primate model, that CD4 T cells are essential to control of initial infection, and that extremely low levels of CD4 T cells can lead to reactivation of latent tuberculosis. These model systems provide an opportunity to identify protective T cell subsets and possibly lead to development of effective vaccines.

Antimycobacterial immune response in household contacts of patients with pulmonary tuberculosis

Helena del Corral[1,2], Lucelly López[1,2], Hanna M. Henao[1,2], T. Martínez[1,2], Nancy Marín[1,2], C. Montes[1,2], Maria. E. Ramírez[1,2], Mauricio Rojas[1,2], Maria P. Arbeláez[1,2], Sara C. París[1,2], Carlos A. Rojas[1,2], Luis Fernando García[1,2]

1 Universidad de Antioquia, Medellín, Colombia.
2 Centro Colombiano de Investigación en Tuberculosis, Medellín, Colombia.

Identification of latent tuberculosis is of particular importance in highly exposed individuals such as household contacts of active TB patients. Tuberculin skin test (TST) has been traditionally used to identify infected individuals; however, its use is hampered by BCG vaccination and environmental mycobateria. IFN-gamma release assays in response to specific *Mycobacterium tuberculosis* (Mtb) antigens have been recommended for contact investigation.

This study evaluates whether IFN-gamma production in response to Mtb antigens correlates with development of TB in household contacts within two years after exposure to a TB case. Sputum smear positive TB patients from Medellín-Colombia were recruited during 2005-2006. Household contacts were interviewed and physically examined. Time and magnitude of exposure to the primary case was documented. IFN-gamma production in whole blood cultures stimulated with CFP, Ag85A, HSPx, and CFP-10 was measured by Elisa. Participants are followed every 3 months and studied for TB when necessary. So far, 366 TB patients and 2060 household contacts have been enrolled. Household contacts are mainly women (58 %), mean age 25, and mean time exposure to the primary case is 2.5 months. At enrollment in the cohort 75 % of the contacts were positive to CFP-10, 86 % to CFP, 40 % to Ag85A, 43 % to HSPx and 65 % had TST diameter 􀂕 10 mm. TST correlated with IFN-gamma production induced by CFP ($p<0.0001$) and CFP-10 ($p<0.0001$). Sixteen TB incident cases have been detected (incidence density: 8.6 cases / 1,000 person-years; rate: 776 / 100,000). Loss to follow up has been less than 1 %.

Experimental model of meningeal tuberculosis in BALB/C mice, evidence of strain ability to disseminate and infect the brain

Rogelio Hernández Pando[1], Martha Guerrero[2], Ingrid Cohen[3], Wellman Ribon Gomez[2], Diana Aguilar, Patricia Acosta, Clara Espitia[3]

1 Experimental Pathology section, Department of Pathology. National Institute of Medical Sciences and Nutrition "Salvador Zubiran", Mexico.
2 University of Bogota, Colombia.
3 Department of Immunology, Biomedical Research Instituto, National University of Mexico.

Cerebral tuberculosis is a severe type of extrapulmonary disease, highly predominant in children. It is thought that meningeal tuberculosis, the most common form of cerebral tuberculosis, begins with respiratory infection followed by early hematogenous dissemination to extrapulmonary sites involving the brain. Host genetic susceptibility factors and specific mycobacterial substrains with distinctive

genotype could be involved in the development of this serious form of tuberculosis. From a comprenhensive epidemiological study in Colombia, we selected three *M. tuberculosis* clinical strains isolated from the cerebrospinal fluid of children with meningeal tuberculosis, and used to infect BALB/c mice by the intratracheal route. These strains showed a distinctive IS-6110 RFLP and spoligotype pattern. The course of infection in terms of strain virulence (mice survival, bacillary loads in lungs and histopathology), bacilli dissemination and extrapulmonary infection (bacilli loads in blood, brain, liver and spleen, brain histopathology), and evocked immune responses (cytokine expression determined by real time) were studied and compared with the laboratory strain H37Rv. Our results showed that these clinical isolates can extensively disseminate by hematogenous route infecting the brain, producing inflammatory nodules in brain parenchima and meningeal inflammation, while H37Rv was totally unable to infect the brain. Proinflammatory and antinflammatory cytokines were expressed along the infection. It is the first study that demonstrate in a well characterized model of pulmonary tuberculosis, the existance of mycobacterial strains with distinctive genotype that have the ability to extensivelly disseminate after respiratory infection and infect the brain.