International Journal of Infectious Diseases 43 (2016) 21–24

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

High levels of anti-tuberculin (IgG) antibodies correlate with the blocking of T-cell proliferation in individuals with high exposure to *Mycobacterium tuberculosis*

Edmond J. Feris [a], Liliana Encinales [b], Carlos Awad [c], Joel N.H. Stern [d,e,f], Inna Tabansky [f], Luis Jiménez-Alvarez [g], Gustavo Ramírez-Martínez [g], Alfredo Cruz-Lagunas [g], Karen Bobadilla [g], Eduardo Márquez [g], Julio Granados-Montiel [h], Tatiana S. Rodriguez-Reyna [i], Marcelo Fernandez-Vina [j], Julio Granados [k], Joaquin Zuñiga [g,*], Edmond J. Yunis [b,h,*]

[a] *Department of Pharmacology and Toxicology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA*
[b] *Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA*
[c] *Hospital Santa Clara, Bogotá, Colombia*
[d] *Departments of Neurology and Science Education, Hofstra Northwell School of Medicine, Hempstead, New York, USA*
[e] *Department of Autoimmunity, The Feinstein Institute for Medical Research, Manhasset, New York, USA*
[f] *The Rockefeller University, New York, USA*
[g] *Department of Immunology, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico*
[h] *Tissue Engineering, Cell Therapy and Regenerative Medicine Research Unit, Instituto Nacional de Rehabilitación, Mexico City, Mexico*
[i] *Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico*
[j] *Department of Pathology, Stanford University School of Medicine, Stanford, California, USA*
[k] *Department of Transplantation, Instituto Nacional de Ciencias Médicas y Nutrición, Mexico City, Mexico*

ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 24 November 2015
Accepted 5 December 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Tuberculosis
Mycobacterium tuberculosis
Latent tuberculosis
Humoral immunity
Anti-tuberculin antibodies
T-cell proliferation

SUMMARY

Objectives: To determine the effect of anti-tuberculin antibodies in the T-cell proliferation in response to tuberculin and Candida antigens in individuals with different levels of tuberculosis (TB) risk.
Methods: Sixteen high-risk TB individuals, 30 with an intermediate TB risk (group A), and 45 with a low TB risk (group B), as well as 49 control individuals, were studied. Tuberculin skin test (TST) results were analyzed and serum levels of antibodies (IgG and IgM) against purified protein derivative (PPD) were measured by ELISA. Tuberculin and Candida antigens were used to stimulate T-cell proliferation in the presence of human AB serum or autologous serum.
Results: High levels of anti-tuberculin IgG antibodies were found to be significantly associated with the blocking of T-cell proliferation responses in cultures stimulated with tuberculin but not with Candida antigens in the presence of autologous serum. This phenomenon was particularly frequent in high-risk individuals with high levels of anti-tuberculin IgG antibodies in the autologous serum when compared to the other risk groups, which exhibited lower levels of anti-tuberculin antibodies.
Conclusions: Although cellular immunity plays a central role in the protection against TB, humoral immunity is critical in the control of *Mycobacterium tuberculosis* infection in high-risk individuals with latent TB infection.

* Corresponding authors.
E-mail addresses: joazu@yahoo.com (J. Zuñiga), edmond_yunis@dfci.harvard.edu (E.J. Yunis).

http://dx.doi.org/10.1016/j.ijid.2015.12.004

1. Introduction

Only 5–10% of patients with a latent tuberculosis infection (LTBI) develop an active TB infection (ATBI).[1] Cellular immunity plays a pivotal role in the control of *Mycobacterium tuberculosis* infection. However humoral responses are also important for protection from *M. tuberculosis*.[1,2] This study group has previously shown that regardless of tuberculin skin test (TST) results, high anti-tuberculin antibody titers are correlated with protection against ATBI, providing a reliable indicator of LTBI.[3]

T-cell responses to tuberculin and Candida antigens were analyzed in the present study and it was found that T-cell proliferation can be modified by anti-tuberculin-specific antibodies. This indicates a significant role of humoral immunity against tuberculin in LTBI.

2. Methods

Sixteen high-risk (HR) TB subjects (spouses, physicians, and significant others), 30 nurses with variable degrees of *M. tuberculosis* exposure considered at intermediate risk of TB (group A), and 45 hospital employees (security, administrative personnel) with a low TB risk (group B) were studied; the mean age of the subjects was 42 ± 11.4 years. Participants were recruited from the Tuberculosis Clinic, Hospital Santa Clara, Bogota, Colombia. Forty-nine healthy controls (mean age 33 ± 13.5 years), with no history of ATBI exposure, were also included. Subjects were recruited between 2002 and 2006 and we did not include immunocompromised individuals, patients using steroids, or patients with neoplasias. The duration of exposure to ATBI patients of the high-risk contacts studied was >6 months for spouses and significant others and >10 years for physicians. The duration of exposure for intermediate and low-risk individuals was >5 years.

The Institutional Review Board of Hospital Santa Clara and of the Dana-Farber Cancer Institute approved the study protocol; subjects provided signed consent. The TST was performed and serum levels of antibodies (IgG and IgM) against purified protein derivative (PPD) were measured as described previously.[3]

T-cell proliferation assays were performed by duplicate in the presence of tuberculin or Candida antigens. Briefly, peripheral blood mononuclear cells (PBMCs; 1×10^5) from 13 HR, 12 group A, and 15 group B individuals were incubated with (1) phytohemagglutinin (2 μg/ml; Sigma-Aldrich, St. Louis, MO, USA); (2) *Candida albicans* (20 μg/ml; Green laboratories, Lenoir, NC, USA), and (3) tuberculin (10 μg/ml; Mycos Research LLC, Loveland, CO, USA) in the presence of human AB serum or autologous serum for 3 days at 37 °C in 5% CO_2. Cells were labeled with 3H-thymidine (1 μCi/well) and the radioactivity was measured. Differences were analyzed with the Student's *t*-test and Chi-square or Fisher's exact method; *p*-values of <0.05 were considered significant.

3. Results

The baseline characteristics of the individuals studied are shown in the **Supplementary Material** (Table S1). The HR group showed a higher frequency of negative TST (>80%) compared to group A (60%, $p = 0.01$) and group B (37%, $p = 0.001$). High serum levels of anti-tuberculin IgG antibodies were observed predominantly in HR individuals (93.7%) when compared to the intermediate risk (group A; 43.3%, $p = 0.003$) and low risk (group B; 26.6%, $p = 0.001$) groups (**Supplementary Material**, Table S2). T-cells isolated from HR subjects with high levels of anti-tuberculin IgG antibodies exhibited higher proliferation after stimulation with tuberculin in the presence of human AB serum when compared to the other groups (**Supplementary Material**, Table S3).

Tuberculin and Candida antigens induced high proliferation, particularly in the HR group (Figure 1, Table 1). All HR subjects (13/13, 100%) exhibited proliferation with both stimulations, whereas 4/15 (26.6%) individuals in group B showed proliferation ($p < 0.05$). To assess the proliferation blocking capacity of anti-tuberculin antibodies, T-cell proliferation was measured in the presence of autologous serum or human AB serum. A significant blocking of T-cell proliferation in cultures stimulated with tuberculin in the presence of autologous serum (presence of anti-tuberculin IgG antibodies) was found. Eleven of the 13 (84.6%) HR individuals exhibited this capacity to block T-cell proliferation in the presence

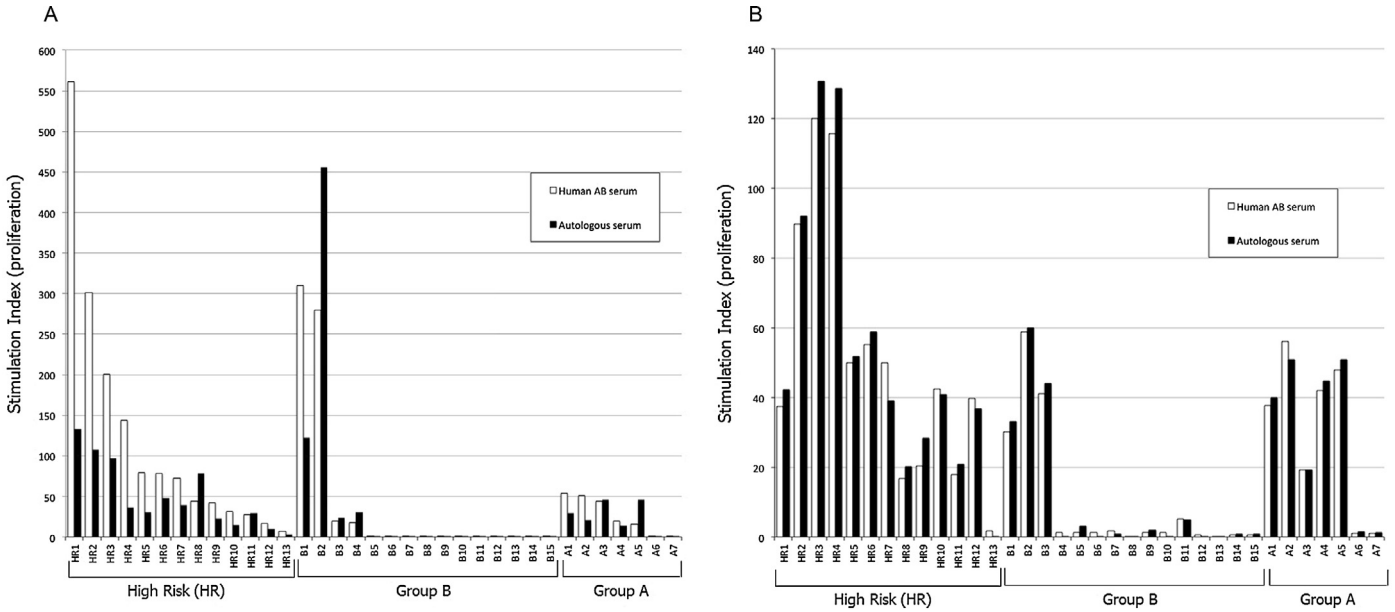

Figure 1. T-cell proliferation induced by tuberculin or Candida antigens in the different TB risk groups. Bar graph depicting the stimulation index (proliferation) levels of T-cells for individuals from each of the groups in the presence of human AB or autologous serum: high-risk (HR), intermediate risk (group A), and low risk (group B). Treatment with autologous serum (with high levels of anti-tuberculin IgG antibodies, black solid bars) provokes the blocking of T-cell proliferation responses after stimulation with tuberculin (Bar graph A), but not with Candida antigen stimulation (Bar graph B). Only one subject from group B and two from group A exhibited blocking activity in the presence of autologous serum.