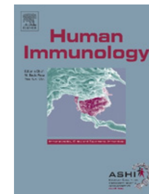
Human Immunology xx (2011) xxx

Contents lists available at ScienceDirect

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



Differential expression of antimicrobial peptides in active and latent tuberculosis and its relationship with diabetes mellitus

Irma Gonzalez-Curiel [a], Julio Castañeda-Delgado [a], Nallely Lopez-Lopez [a], Zaida Araujo [b], Rogelio Hernandez-Pando [c], Benjamin Gandara-Jasso [a,d], Noe Macias-Segura [a], Antonio Enciso-Moreno [a], Bruno Rivas-Santiago [a,*]

[a] Unidad de Investigación Médica, Zacatecas, Instituto Mexicano del Seguro Social, Zacatecas, Mexico
[b] Laboratorio de Inmunología de Enfermedades Infecciosas, Instituto de Biomedicina, Universidad Central de Venezuela, Caracas, Venezuela
[c] Departamento de Investigación en Patología, Instituto Nacional de Ciencias Médicas y Nutricion SZ, Mexico City, Mexico
[d] Coordinacion de Investigacion Medica, Servicios de Salud de Zacatecas, Zacatecas, Mexico

ARTICLE INFO

Article history:
Received 7 December 2010
Accepted 31 March 2011
Available online 20 April 2011

Keywords:
Tuberculosis
Antimicrobial peptides
Diabetes

ABSTRACT

Tuberculosis (TB) is one of the most important infectious diseases, causing 1.8 million deaths annually worldwide. This problem has increased because of the association with human immmunodeficiency virus and diabetes mellitus type 2, mainly in developing countries. In the past few years it has been highlighted the significance of antimicrobial peptides in the immunopathogenesis of TB *ex vivo* and in experimental models studies. In this study we analyzed the expression of CAMP, DEFA1, DEFB4, and DEFB103A in patients with latent TB and progressive TB with and without comorbidity with diabetes mellitus type 2. Antimicrobial peptide gene expression increased during progressive TB, which could be used as a biomarker for reactivation. By contrast, patients with diabetes mellitus type 2 have lower antimicrobial peptides gene expression, suggesting that the lack of its proper production in these patients contribute to enhance the risk for TB reactivation.

© 2011 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

1. Introduction

Despite great efforts of health care systems worldwide to abolish deaths caused by tuberculosis (TB), World Health Organization still reports 1.8 million deaths annually caused by *Mycobacterium tuberculosis* (Mtb), which is the causative agent of TB; the number of new cases continues to grow, approaching 10 million in 2010 [1,2]. Moreover, there is evidence that diabetes is an important risk factor for active TB: the incidence of TB is two to five times higher in patients with diabetes than in those without diabetes [3,4]. With rising rates of obesity and diabetes, particularly in low-income and middle-income countries where high rates of TB remain, there is concern that diabetes mellitus type 2 poses a threat to global TB control.

Mycobacterium tuberculosis infection is mainly acquired by inhalation of infecting aerosol droplets, which are dispersed by coughing of persons with active pulmonary disease. However, the infection infrequently progresses directly to active disease, and is often contained as dormant or latent infection that can persist or reactivate many years later. Tuberculosis chemotherapy can also contain the disease, but patients remain with latent infection that is capable of causing disease relapse [5].

Antimicrobial peptides (AMPs) represent an important part of the innate immunity response to several infectious diseases [6]. In mammals, AMPs are present mainly in phagocytic cells of the immune system for the killing of engulfed or invasive bacteria [7]. In addition to a direct antimicrobial effect, AMPs possess the ability to modulate the immune response through a variety of mechanisms, such as chemotaxis, activation of immature dendritic cells, and cytokine induction [8–10]. Over the past 2 decades, several families of AMPs have been described as existing in many living organisms. In human beings, there are three families of AMPs, including defensins, cathelicidins, and histatins [11–13]. Although there are several antimicrobial peptides, only a few of them have been related to tuberculosis infection, mainly during early infection, either in vitro or in murine models; these peptides are human β-defensin (HBD)–2, HBD-3, human neutrophil peptide (HNP), and cathelicidin LL-37 [14–23]. However, the expression of these peptides has not been explored in human beings either during TB latent infection or during active tuberculosis. We sought to determine the differential expression of LL-37 (CAMP), HNP-1 (DEFA1), HBD-2 (DEFB4), and HBD-3 (DEFB103A) during latent infection and active pulmonary TB, and whether the comorbidity with DM2 decreases AMPs gene expression which could lead to MTb progression.

2. Subjects and methods

2.1. Subjects recruitment and stratification

Total populations of 60 adults of both genders, 22 to 65 years of age, were enrolled in this study under a strict clinical and labora-

* Corresponding author.
E-mail address: rondo_vm@yahoo.com (B. Rivas-Santiago).

0198-8859/11/$32.00 - see front matter © 2011 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2011.03.027

tory criteria. Subjects were stratified mainly into two groups. The first group comprised patients diagnosed with diabetes mellitus type 2 (DM2); the other group comprised individuals without diabetes mellitus type 2 (NoDM2). Individuals with DM2 were recruited according to fasting plasma glucose levels of at least 126 mg/dl, and random plasma glucose of at least 200 mg/dl and 2-hour plasma glucose of at least 200 mg/dl during an oral glucose tolerance test. All individuals also had no evidence of another specific type of diabetes, and all patients were non–insulin dependent. Only patients with known disease duration of >1 year according to clinical files and recent HbA1c $\geq$ 7.0% were enrolled [24], Each group was subdivided into three clinical groups of 10 individuals each, according to their tuberculosis infection status: (1) subjects not infected with *Mycobacterium tuberculosis* (control subjects); (2) subjects with latent TB infection (LTB); and (3) subjects with progressive TB (PTB) (Fig. 4). Approval to perform venipunctures was given by the Institutional boards at the Mexican Institute for Social Security (IMSS) and the General Hospital #1 of Zacatecas. Written informed consent was obtained from all study participants according to the guidelines of the National Committee of Ethics at IMSS.

2.2. Inclusion and exclusion criteria

After the written informed consent was obtained from each patient, according to the Declaration of Helsinki, each subject was interviewed, examined clinically, and a chest radiograph was obtained. To determine progressive tuberculosis, sputum samples were collected for acid-fast bacilli (AFB) smear and *M tuberculosis* culture tests. Broncheoalveolar washing specimen was obtained in those subjects unable to expectorate. In the case of *M tuberculosis* culture or AFB smear tests being negative in individuals suspected of having active TB, an additional *M tuberculosis* polymerase chain reaciton (PCR) IS 6110 test was considered for diagnosis. Subjects were included in the NoDM2-PTB or in the DM2/PTB group only when at least two of the three tests mentioned above were positive. For latent infection in patients with or without DM2 diagnosis, the cutaneous test for tuberculin (TST) was conducted using the Mantoux method, using Tuberculin Purified Protein Derivative TUBERSOL (5 TU/0.1 ml; Senofi-Pasteur, Toronto, Ontario, Canada), and the *in vitro* T-cell interferon-γ (IFN-γ) release assay QuantiFERON TB-Gold test (Cellestis, Victoria, Australia) were used in accordance with manufacturers' guidelines. For the anergy test, Candidine (Indre, Mexico City, Mexico) (1:1000, 0.1 ml) was administered by intradermic injection into the foreside of the forearm. The size (in millimeters) of induration was recorded. Healthy individuals (DM2/control NoDM2/Control) were enrolled if they showed no clinical disease manifestation, negative TST, negative QuantiFERON TB-Gold test, or positive candidine test and without diagnostic for DM2. For the DM2/Control group, the same criteria were taken as those for noDM2/control group. All inclusion and exclusion criteria are summarized in Fig. 4.

2.3. Sample collection, RNA isolation, and reverse transcription

Peripheral blood was drawn directly in two to three PAXgene tubes (PreAnalytix, Hombrechtikon, Switzerland). After mixing the blood by inverting 10 times, PAX tubes were left at room temperature for 2 hours, then stored at -20°C overnight and after that at -80°C until use.

Total RNA was extracted and purified using PAXgene Blood RNA kit, and contaminating DNA was removed by on-column DNase digestion according the manufacturer's directions (PreAnalytix, Hombrechtikon, Switzerland). RNA content and purity were determined by measuring the absorbance at 260 nm and 280 nm, using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Total RNA was enriched and concentrated by eliminating of tRNA, 5S rRNA and 5.8 rRNA molecules using RNeasy MiniElute CleanUp System (Qiagen, Mexico City, Mexico). Then, 2 μg of enriched RNA was treated to remove the majority of unwanted globin mRNA from whole-blood total RNA using GLOBINclear Human System (Ambion, Applied Biosystems, Victoria, Australia), in accordance with the manufacturer's instructions. To generate cDNA, 250 ng of globin transcripts-free RNA was reverse transcribed using random and oligo dT primers and SuperScript II Reverse Transcriptase (Invitrogen, Carslbad, CA, USA).

2.4. Gene expression analysis determined by real-time PCR

Real-time PCR was performed using the Light Cycler 2.0 (Roche, Manheim, Germany), Light Cycler Taqman mastermix, and the specific probe for each gene selected from the Universal Probe Library (Roche, Germany). All primers were designed with Universal Probe Library software from Roche (Table 1). The relative expression of each sample was calculated using human mRNA HPRT as reference gene in all experiments and the $\Delta\Delta$CT method as described previously [25]. This method is based on the expression levels of a target gene (AMPs) versus one reference gene (HPRT) for comparison between the control group and target group.

2.5. Statistical analysis

Normality of the data from the reverse transcription– quantitative polymerase chain reaction (RT-qPCR) assays were analyzed using a Kolmogorov–Smirnov normality test for each data set. Once done, the nonparametric multiple comparison test of Kruskal–Wallis was used to identify differences among groups. In the case of statistical significance ($p < 0.05$), a Dunn post test was performed to identify such differences. For the analysis of the clinical characteristics of the individuals, normality was verified using the same test, after which a nonparametric multiple comparison test of Kruskal–Wallis was used to identify differences among groups. Two-sided p values of <0.05 were considered statistically significant. Statistical analyses were performed by the GraphPad Prism software for Windows (GraphPad Software version 5.02; San Diego, CA).

3. Results

3.1. Individual clinical data analysis

To rule out the possibility that any clinical variant besides Tb or DM2 might interfere with the production of antimicrobial peptides, the main clinical parameters were compared between groups (Table 2. Age comparisons among the different groups did not show statistical difference ($p = 0.067$). Hemoglobin and cholesterol lev-

Table 1
Sequence of primers and taqman probes used for RT-qPCR assays

Gene name	Peptide	Probe sequence	Right primer	Left Primer
DEFA1	HNP-1	CAG GAG AA	TCC CTG GTAGAT GCA GGT TC	CTT GGC TCC AAA GCA TCC
DEFB103A	HBD-3	CTG CCT TC	GAG CAC TTG CCG ATC TGT TC	CAG AAA TAT TAT TGC AGA GTC AGA GG
DEFB4	HBD-2	TGT GGC TG	GAG GGA GCC CTT TTC TGA ATC	GTC TCC CTG GAA CAA AAT GC
CAMP	LL-37	TCC AGG TC	GTC TGG GTC CCC ATC CAT	TCG GAT GCT AAC CTC TAC CG
HPRT	HPRT	GCT GAG GA	CGA GCA AGA CGT TCA GTC CT	TGA CCT TGA TTA TTT TGC ATA CC

HBD, human beta-defensin; HNP, human neutrophil peptide; HPRT, hypoxanthine-guanine phosphoribosyltransferase.

Table 2
Clinical characteristics of study population

	No DM2			DM2			*p* Value
	Control group ($n = 10$)	LTB ($n = 10$)	PTB ($n = 10$)	Control group ($n = 10$)	LTB ($n = 10$)	PTB ($n = 10$)	
Age (y)	33.67 ± 13.19	38.3 ± 13.7	42.11 ± 14.25	55.9 ± 6.62	52.7 ± 6.1	59.8 ± 5.6	0.067
Gender (Male/Female)	8/2	1/9	4/6	4/6	3/7	3/7	—
PPD (mm)	3.7 ± 3.28	16.1 ± 5.52	NA	0 ± 0	22.1 ± 6.2	NA	< 0.0001[a]
Hb (g/dl)	15.7 ± 1.7	14.4 ± 1.5	13.9 ± 2.8	15.2 ± 2.5	14.4 ± 1.2	12 ± 2.5	0.0346
DM2 evolution (y)	NA	NA	NA	11.8 ± 7	7.6 ± 4.9	14.5 ± 11.8	0.2142
Glucose (mg/dl)	86.6 ± 6.9	91.3 ± 6.9	87.1 ± 18	231.1 ± 98.1	172.4 ± 71.3	176.3 ± 67.4	< 0.0001[a]
Cholesterol (mg/dl)	186 ± 12.4	183.2 ± 43.2	174.6 ± 39	200.8 ± 41.5	208.4 ± 55.7	139.6 ± 39.2	0.1676
Triglycerides (mg/dl)	149.9 ± 100.2	109.4 ± 31.8	114.2 ± 44.5	229.7 ± 61.5	179.6 ± 86.9	115 ± 45.4	0.0024[a]
Hb1ac (%)	—	—	—	7.7 ± 1.18	6.51 ± 1.84	6.2 ± 0.11	0.1713
Smoking habit	4/10	5/10	6/10	3/10	5/10	1/10	—
Alcoholism	0/10	0/10	0/10	0/10	0/10	0/10	—
Arterial hypertension	1/10	0/10	0/10	5/10	5/10	0/10	—

DM2, type 2 diabetes mellitus; Hb, hemoglobin; Hb1ac, glycated hemoglobin; LTB, latent tuberculosis infection; NA, not applicable; PPD, purified protein derivative; PTB, progressive pulmonary tuberculosis.
Data shown as median ± SD; normality of the data was checked with a Kolmogorov–Smirnov normality test.
[a]Statistically significant difference for a non-parametric Kruskal–Wallis multiple comparisons test.

els were similar between the different groups showing non-statistical significance ($p = 0.0346$ and $p = 0.1676$, respectively). Nevertheless, triglyceride levels were higher in DM2 patients, with a statistical difference between groups ($p = 0.0024$).

As expected, there were differences among the different groups in parameters related to tuberculosis, such as PPD, or to DM2, such as glucose levels. Noteworthy, in the DM2 group, there was no difference in the time of evolution of this disease.

3.2. *Differential expression of antimicrobial peptides in NoDM2 subjects*

Quantitative analysis of CAMP gene expression from blood samples determined by real-time PCR revealed that there was no significant difference between the healthy subjects and the latently infected group. A significant increase of CAMP expression was seen in progressive tuberculosis than in LTB or healthy subjects ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 1A). When DEFA1 gene expression was analyzed, there was statistical sig-

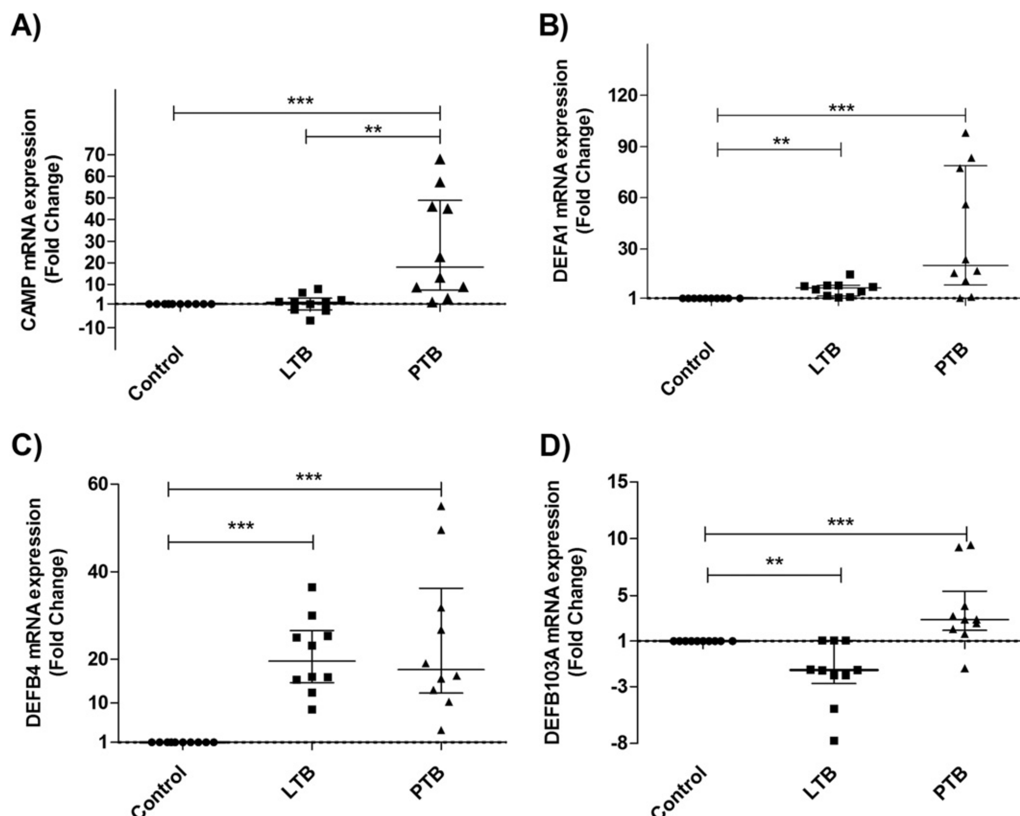


Fig. 1. Differential expression of antimicrobial peptides of patients NoDM2. Analysis of genetic expression of (a) CAMP (b), DEFA1 (c), DEFB4, and (d) DEFA103A in blood during latent tuberculosis (TB) (LTB) and progressive TB (PTB) by real time RT-PCR. Data are represented as median with interquartile range. Statistics were calculated by Kruskal–Wallis test. In each experimental group, $n = 10$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group.

ARTICLE IN PRESS

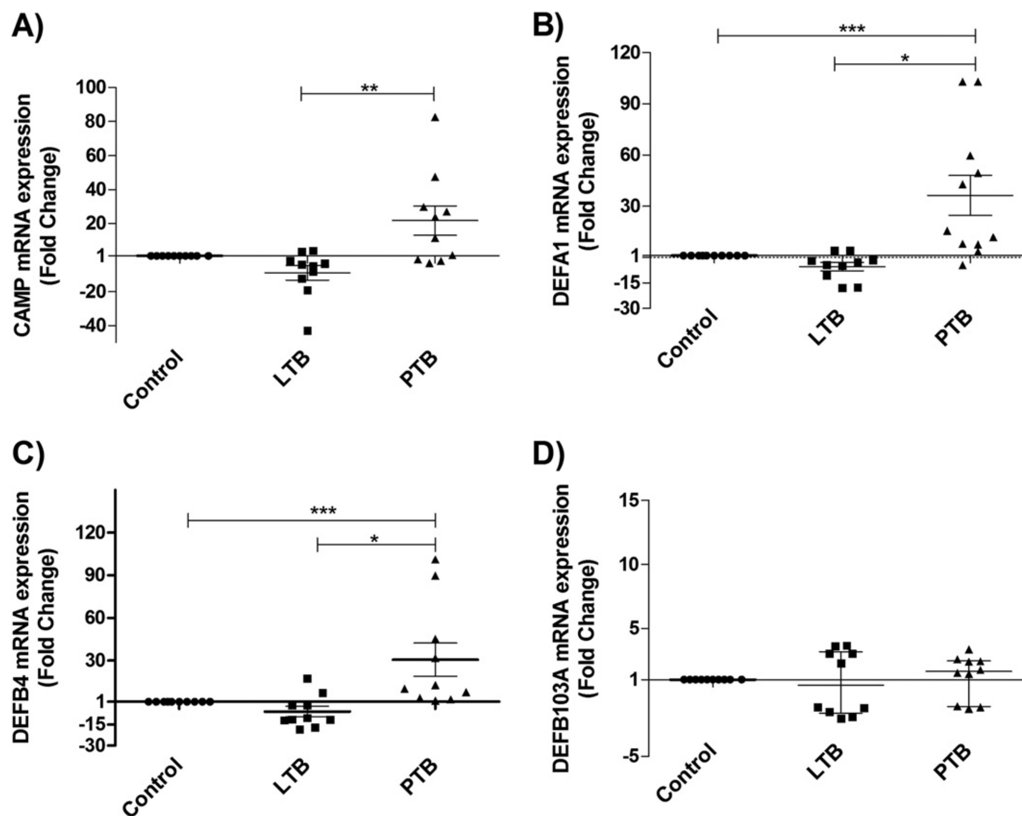


Fig. 2. Differential expression of antimicrobial peptides of patients DM2. Analysis of genetic expression of (a) CAMP (b) DEFA1, (c) DEFB4, and (d) DEFA103A in blood during DM2 with latent TB (LTB) and progressive TB (PTB) by real-time RT-PCR. Data are represented as median with interquartile range. Statistics were calculated by Kruskal–Wallis test. In each experimental group, $n = 10$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group.

nificance only between the PTB and the control group ($p < 0.001$), though there was a difference between LTB and PTB, this was not statistically significant (Fig. 1B). DEFB4 gene expression was very high in the PTB group, showing significant difference with the LTB group and control subjects ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 1C). Similar results were obtained with DEFB103A gene expression (Fig. 1D).

3.3. Differential expression of antimicrobial peptides in DM2 subjects

Patients with DM2 have greater susceptibility to develop TB [3,4]; therefore we sought to determine whether in patients either with latent infection or with progressive or active TB, the main antimicrobial peptides gene expression was affected in blood samples. When CAMP gene expression was analyzed by RT-qPCR, greater fold changes were seen only in PTB group; some of these patients reach fold changes of 80 (Fig. 2A), showing statistical significance when these were compared with those in the LTB group ($p < 0.01$). DEFA1 gene expression analysis showed that expression was augmented only in the PTB reaching fold changes >60 (Fig. 2B) and showing statistical significance when compared with control group and LTB group ($p < 0.05$ and $p < 0.001$, respectively). Comparable results were seen with the DEFB4 gene expression (Fig. 2C), PTB group showed significant higher levels when compared with the control group ($p < 0.001$) or with the LTB group ($p < 0.05$). Finally, in this DM2 group, DEFB103A did not show difference among control group, LTB and PTB (Fig. 2D).

3.4. DM2 affects antimicrobial peptide expression during Tb infection

Because most of the antimicrobial peptides analyzed in this study are highly expressed during PTB either in DM2 or in NoDM2 subjects, we compared whether, in patients with PTB, the fact of comorbidity with DM2 decreased antimicrobial peptides gene expression through the comparison between each NoDM2 group against its counterpart in the DM2 group. When CAMP gene expression was analyzed between the DM2 and NoDM2 groups, all the DM2 subgroups showed significant lower CAMP gene expression when compared with the NoDM2 group ($p > 0.001$) (Fig. 3A). When DEFA1 gene expression was analyzed, we found differences only between the DM2-LTB and NoDM2-LTB individuals (Fig. 3B); the other groups did not show statistical difference, while DEFB4 and DEFA103A showed similar gene expression (Fig. 3C, 3D, respectively) in all subgroups. When were compared NoDM2 control group with DM2 control group, NoDM2-LTB with DM2-LTB and NoDM2-PTB with DM2-PTB all showed similar statistical significance ($p < 0.001$).

4. Discussion

In the last few years, it has been documented the importance of antimicrobial peptides in the immunopathogenesis of TB [6,14,15,17–20,26–29]. In fact, some authors suggest that cathelicidin might be a key molecule for the control of TB during the primary infection [6,16,17,26], whereas other studies suggest that β-defensins are the main molecules used by epithelial cells to eliminate bacteria and avoid invasion [19–20]. By contrast, as natural killer cells, $\gamma\delta$ T cells, CD8^{+} T cells, and neutrophils actively participate during *M tuberculosis* infection by controlling mycobacteria growth, and all of these cells produce HNP-1 [18,27,30–33], some authors have suggested HNP-1 as a potential molecule to eliminate mycobacteria [21-23,34-36]. However, it is quite possible that all AMP work together to eliminate mycobacteria during primary infection by their direct antimicrobial effect and immunoregulatory activities. Moreover, it has been suggested that β-defensins could contribute to maintain latent infection [19,28]. Thus,

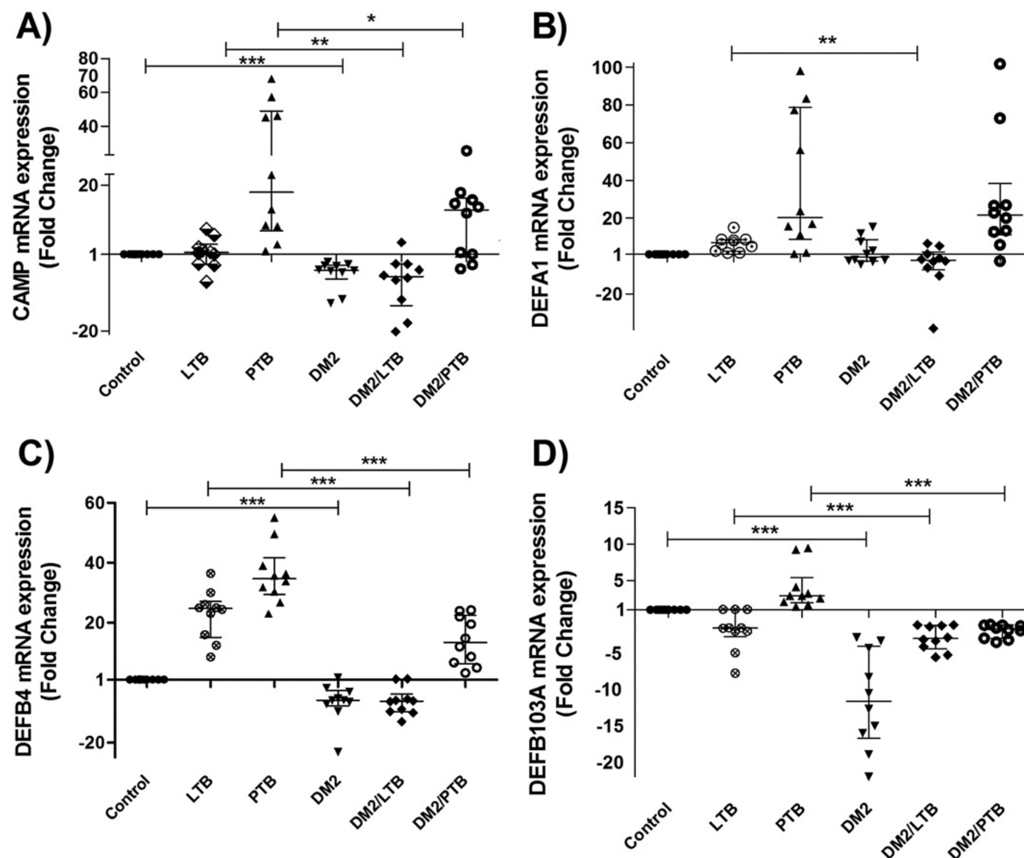


Fig. 3. DM2 downregulation expression of antimicrobial peptides during latent tuberculosis infection. Analysis of genetic expression of (a) CAMP, (b) DEFA1, (c) DEFB4, and (d) DEFA103A by real-time RT-PCR. Data are represented as median with interquartile range. Statistics were calculated by Kruskal–Wallis test. In each experimental group, $n = 10$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group.

we analyzed antimicrobial peptide differential gene expression in blood cells from patients with active TB compared with those in subjects with latent infection, the main antimicrobial peptide gene expression in comparison with that healthy individuals. It is important to mention that the subject recruitment was carried out with very strict inclusion and exclusion criteria, without any clinical parameters that indicated significant differences among the groups; thus all findings of the gene expression analysis should be due to TB infection instead of to some other clinical entity. Because hypoxanthine phosphoribosyltransferase plays a central role in the generation of purine nucleotides through the purine salvage pathway, we sought to determine whether this reference gene expression was altered in diabetic individuals. Our CP analysis according to a method previously reported [25] of analysis reference genes between groups showed no statistical difference (data not shown); thus HPRT gene expression between groups was not a factor for variations during gene expression analysis.

CAMP gene expression was only significantly augmented in the group of progressive TB, whereas in latent TB was similar to the control group. In previous studies, we had similar results in murine models with progressive disease and latent infection; high production of CRAMP (Cathelicidin-Related Antimicrobial Peptide), which is the ortholog of human cathelicidin, was seen during the late phase of progressive disease, whereas in the latent infection model CRAMP did not change [26].

Our results also agree with those of other investigators, who have demonstrated high concentrations of cathelicidin in serum from patients with acid-fast bacilli sputum smear–positive [29]. The association between blood sample cathelicidin gene expression and clinical signs of disease severity and immune activation in TB patients suggests that cathelicidin gene expression levels may serve as a marker of systemic immune activation and the inflammatory milieu of the host. Cells of the immune system, such as neutrophils and monocytes, are known sources of cathelicidin [18]; thus, elevation of peripheral leukocytes in the setting of infection may lead to higher circulating concentrations of cathelicidin.

Both HNP-1 and HBD-3 peptides showed gene expression in latent infection similar to that in the control group; nevertheless, during active infection a considerable increase was seen. As in cathelicidin, these may be related to the increase of cell counts during infection.

Regarding HBD-2, higher levels were detected during latent infection, which correlates with our previous results in a murine model of chronic infection similar to latent infection [19,28], suggesting that perhaps this kind of peptide contributes to maintain dormant bacilli. In agreement with our previous results in an experimental mouse model, we expected an important decrease of this peptide during progressive infection [19]. However, a slight HBD-2 expression decrease was seen during progressive TB in comparison with latent infection. This difference could be explained because in the experimental murine model, mRNA was isolated from the lung, where epithelial cells are the main β-defensin-2 source, whereas in the bloodstream this defensin is rarely expressed by some phagocytic cells [20,37].

It has been documented that particularly in low-income and middle-income countries where high rates of TB remain, DM2 is a main risk factor for developing active TB [4]. Thus, we sought to determine whether antimicrobial peptide gene expression in DM2 patients, either with latent TB or with progressive TB, behaves similarly to that in patients exclusively with TB infection. Our

	DM2 Group			Non DM2 Group		
Clinical Subgroup	LTB	PTB	CONTROL	LTB	PTB	CONTROL
PPD Skin test (≥10 mm)	+	Non required	-	+	Non required	-
QFT-TB Gold (≥0.35 IU/ml IFN-γ)	+	+	-	+	+	-
Sputum Smear Positive for AFB	-	+	-	-	+	-
Mycobacterial culture	-	+	-	-	+	-
Clinical symptoms	-	+	-	-	+	-
Chest X-ray	-	+	-	-	+	-
Candidine (≥5 mm)			+			+
COMBE			-			-

Fig. 4. Inclusion and exclusion criteria. Figure shows the different inclusion and exclusion criteria taken to differentiate rigorously each group and the different subgroups.

results showed the same pattern of CAMP, DEFA1, and DEFB103A gene expression in NoDM2 patients than in DM2 patients. These results may suggest that these antimicrobial peptides, mainly cathelicidin, could be used as a biomarker to indicate TB progression in both DM2 and NoDM2 patients. Intriguingly, levels of DEFB4 were lower in DM2 individuals with latent infection than in NoDM2 patients, which may suggest that these patients could be more susceptible to developing active TB, as previous experimental results suggest that β-defensin-2 may contribute to maintain latency [19,28], probably by the binding and inactivation of ftsZ factor, a crucial protein involved in the control of bacterial proliferation [19,28]. Thus, it is plausible to propose that the lower HBD-2 production in DM2 patients could contribute to TB progression, also facilitating dormant bacilli reactivation, as these patients do not have a proper DEFB4 expression.

Another interesting finding was the lower expression of all the AMP studied in DM2, either in the LTB or in the PTB group, compared with the NoDM2 TB groups, suggesting that this could be a factor contributing to the high risk of diabetic patients developing TB, particularly reactivation of latent infection.

A potential limitation of the current study is the low number of individuals per group. Nevertheless, the inclusion and exclusion criteria were strict in selecting each individual from each group. Even when we considered that our groups represented true PTB, latent-infected and noninfected individuals, to evaluate the suitability of antimicrobial peptides as biomarkers, further studies with a higher number of individuals are needed.

Acknowledgments

We acknowledge Nicolas Hernandez, Leonor Enciso, Ema Valenzuela and Yolanda García from the UIMZ-IMSS for their valuable help for collecting samples. This work was supported by the National Council of Science and Technology in Mexico (CONACYT; grant CB-2007-01-82424, FOMIX-Zacatecas- 2008-C01-109189, CONACYT-FOSSIS 2008-87863 and CONACYT FS SALUD 2005-02-1444).

References

[1] Dye C, Williams BG. The population dynamics and control of tuberculosis. Science 2010;328:856–62.
[2] World Health Organization. Global Tuberculosis Control Report. Geneva, World Health Organization, 2009.
[3] Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: Convergence of two epidemics. Lancet Infect Dis 2009;9:737–44.
[4] Dooley KE, Tang T, Golub JE, Dorman SE, Cronin W. Impact of diabetes mellitus on treatment outcomes of patients with active tuberculosis. Am J Trop Med Hyg 2009;80:634–40.
[5] Wallis RS, Pai M, Menzies D, Doherty TM, Walzl G, Perkins, et al. Biomarkers and diagnostics for tuberculosis: Progress, needs, and translation into practice. Lancet 2010;375:1920–8.
[6] Rivas-Santiago B, Serrano CJ, Enciso-Moreno JA. Susceptibility to infectious diseases based on antimicrobial peptide production. Infect Immun 2009;77: 4690–6.
[7] Rivas-Santiago B, Sada E, Hernandez-Pando R, Tsutsumi V. Antimicrobial peptides in the innate immunity of infectious diseases. Salud Públ Mex 2006;48: 62–72.
[8] Bowdish DM, Davidson DJ, Hancock RE. Immunomodulatory properties of defensins and cathelicidins. Curr Top Microbiol Immunol 2006;306:27–33.
[9] Biragyn A. Defensins—non-antibiotic use for vaccine development. Curr Protein Pept Sci 2005;6:53–61.
[10] Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov, et al. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. Science 2002;298:1025–32.
[11] Brown KL, Hancock RE. Cationic host defense (antimicrobial) peptides. Curr Opin Immunol 2006;18:24–32.
[12] Hale JD, Hancock RE. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. Expert Rev Anti Infect Ther 2007;5:951–60.
[13] Lehrer RI, Ganz T. Cathelicidins: A family of endogenous antimicrobial peptides. Curr Opin Hematol 2002;9:18–25.
[14] Kisich KO, Heifets L, Higgins M, Diamond G. Antimycobacterial agent based on mRNA encoding human beta-defensin 2 enables primary macrophages to restrict growth of Mycobacterium tuberculosis. Infect Immun 2001;69: 2692–9.
[15] Kisich KO, Higgins M, Diamond G, Heifets L. Tumor necrosis factor alpha stimulates killing of Mycobacterium tuberculosis by human neutrophils. Infect Immun 2002;70:4591–9.
[16] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik S, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311:1770–5.
[17] Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. J Immunol 2007;179:2060–9.
[18] Rivas-Santiago B, Hernandez-Pando R, Carranza C, Juarez E, Contreras JL, et al. Expression of cathelicidin LL-37 during Mycobacterium tuberculosis infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. Infect Immun 2008;76:935–43.
[19] Rivas-Santiago B, Sada E, Tsutsumi V, Aguilar-Leon D, Contreras JL, Hernandez-Pando R, et al. beta-Defensin gene expression during the course of experimental tuberculosis infection. J Infect Dis 2006;194:697–703.
[20] Rivas-Santiago B, Schwander SK, Sarabia C, Diamond G, Klein-Patel ME, Hernandez-Pando. {Human, in beta}-defensin 2 is expressed and associated with

ARTICLE IN PRESS

Mycobacterium tuberculosis during infection of human alveolar epithelial cells. Infect Immun 2005;73:4505–13.
[21] Sharma S, Khuller G. DNA as the intracellular secondary target for antibacterial action of human neutrophil peptide-I against Mycobacterium tuberculosis H37Ra. Curr Microbiol 2001;43:74–83.
[22] Sharma S, Verma I, Khuller GK. Antibacterial activity of human neutrophil peptide-1 against Mycobacterium tuberculosis H37Rv: In vitro and ex vivo study. Eur Respir J 2000;16:112–20.
[23] Sharma S, Verma I, Khuller GK. Therapeutic potential of human neutrophil peptide 1 against experimental tuberculosis. Antimicrob Agents Chemother 2001;45:639–47.
[24] Diagnosis and classification of diabetes mellitus. Diabetes Care 2006;29(Suppl 1):S43.
[25] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25: 402–10.
[26] Castaneda-Delgado J, Hernandez-Pando R, Serrano CJ, Aguilar-Leon D, Leon-Contreras J, et al. Kinetics and cellular sources of cathelicidin during the course of experimental latent tuberculous infection and progressive pulmonary tuberculosis. Clin Exp Immunol 2010;161:542–50.
[27] Martineau AR, Newton SM, Wilkinson KA, Kampmann B, Hall BM, Nawroly. Neutrophil-mediated innate immune resistance to mycobacteria. J Clin Invest 2007;117:1988–98.
[28] Rivas-Santiago B, Contreras JC, Sada E, Hernandez-Pando R. The potential role of lung epithelial cells and beta-defensins in experimental latent tuberculosis. Scand J Immunol 2008;67:448–55.
[29] Yamshchikov AV, Kurbatova EV, Kumari M, Blumberg HM, Ziegler TR, Ray S, et al. Vitamin D status and antimicrobial peptide cathelicidin (LL-37) concentrations in patients with active pulmonary tuberculosis. Am J Clin Nutr 2010;92: 603–10.
[30] Barrios-Payan J, Aguilar-Leon D, Lascurain-Ledezma R, Hernandez-Pando R. Neutrophil participation in early control and immune activation during experimental pulmonary tuberculosis. Gac Med Mex 2006;142:273–9.
[31] Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom, et al. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood 2000; 96:3086–94.
[32] Lehrer RI. Multispecific myeloid defensins. Curr Opin Hematol 2007;14:16–25.
[33] Trabattoni D, Caputo SL, Maffeis G, Vichi F, Biasin M, Pierotti, et al. Human alpha defensin in HIV-exposed but uninfected individuals. J Acquir Immune Defic Syndr 2004;35:455–63.
[34] Soehnlein O. Direct and alternative antimicrobial mechanisms of neutrophil-derived granule proteins. J Mol Med 2009;87:1157–65.
[35] Soehnlein O, Kai-Larsen Y, Frithiof R, Sorensen OE, Kenne E, Scharffetter-Kochanek, et al. Neutrophil primary granule proteins HBP and HNP1-3 boost bacterial phagocytosis by human and murine macrophages. J Clin Invest 2008; 118:3491–9.
[36] Tan BH, Meinken C, Bastian M, Bruns H, Legaspi A, Ochoa M, et al. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. J Immunol 2006;177:1864–70.
[37] Duits LA, Ravensbergen B, Rademaker M, Hiemstra PS, Nibbering PH. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunology 2002;106:517–25.