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Biochemical and Immunological Characterization of the *Mycobacterium tuberculosis* 28 kD Protein

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1. Introduction

Tuberculosis is currently a worldwide public health problem [Tiruviluamala, LB., 2002. World Health Organization, 2005]. Up to 33% of the world population may be infected due to the accelerated resurgence of the disease showing high resistance to drugs, which has been favored by the HIV epidemic [De Cock, KM., 2002], and to individual levels of susceptibility [Cooke, GS., 2001] and predisposition [Ogus, AC., 2004]. The unequal protection conferred by the BCG vaccine, which may range from 0% to 80% [Kipnis, A., 2005] depending on the individual, has led to the search for new vaccination strategies. Recent studies have focused on the identification of immunoprotective bacterial antigens [Zhang, Y., 2004].

Evidence has been forwarded which suggests that protective immunity against TB is closely related to the presence of CD4+ cells [Zhang, M. 1995]. Certain antigenic fractions of MW less than 10 kD with immunoprotective activity have been described [Boesen, H., 1995]. Other fractions of MW 45-64 and 61-80 kD [Harris, D.P., 1993] as well as a heat shock protein of 65 kD transfected to macrophages have been found to induce protective immunity [Cooper, A., 1995]. Moreover, CD8+ cells are known to lyse macrophages which express TB bacillus peptides on their surface [Flynn, JL., 1992]. Some bacillus proteins which induce protection are those of 30-31 and 19 kD [Méndez-Samperio, P., 1995], as well as the 38 kD fraction [Elinos, C.M., 2009]. Such observations suggest that the living microorganism releases certain protective and other potent immunosuppressive molecules. The specific activity of these molecules must therefore be determined before they can be suggested as vaccines.

The present study focused on a 28kD protein (Gp28). This fraction had not been characterized yet but is known to be immunogenic since it stimulates the production of

CD4+, CD8+ and TCRαβ, which in turn produce IL-2 and INFγ in healthy individuals [Elinos, C.M., 2009]. The present work determined certain biochemical and immunological characteristics of Gp28 obtained from the cultured strain H37Rv of *Mycobacterium tuberculosis*. A total protein extract (TPE) was obtained from which Gp28 was subsequently isolated. Anti-TPE hyper-immune serum (HIS) and monospecific polyclonal anti-p28 serum were prepared and used to determine p28 purity; as well as the number of peptides that form the protein; to identify the dominant immunoglobulin (IgG2a); to search for mannose-free radicals and classify it as a glycoprotein (Gp28); to determine the percentage of sugars that recognize the anti-Gp28 antibody, and the N-terminal amino acids, thus establishing the predictive nucleotides which form part of the gene. An *M. tuberculosis* strain H37Rv library was used to isolate the gene and confirm DNA purity. Studies were performed by analyzing phenotypic changes in peripheral mononuclear blood cells (PMBC) induced by Gp28 protein obtained from healthy subject involving the production of CD4+ helper T cells [van Crevel, R., et al. 2000] and CD8+ cytotoxic/suppressor cells [Lazarevic, J., 2002] in high risk healthy staff from the National Institute of Respiratory Diseases (INER) in Mexico, and from four TB patients.

2. Materials and methods

2.1 *M. tuberculosis* culture

Bacteria were sown in the Proskawer and Beck synthetic culture medium modified by Youmans (PBY) [Youmans, G.P., 1949], which was incubated for 6 weeks at 37°C to obtain a colony monolayer that completely covered the surface of the liquid culture medium.

2.2 *M. tuberculosis* Total Protein Extract (TPE)

The culture medium was sterilized by filtering through: 1) Whatman sterile #40 filter paper; 2) 1.2 μm micropore filter; 3) 0.45 μm micropore filter; 4) 0.22 μm micropore filter. A sterile filtrate was obtained and added with solid $(NH_4)_2SO_4$

to precipitate proteins and thus obtain the total protein extract (TPE) of *M. tuberculosis*. The TPE was then dialized with water.

2.3 *M. tuberculosis* 28 kD protein purification

The TPE was treated with the method described by Seibert [Seibert, F. B., 1949]. The acid-alcoholic fraction 2 was obtained enriched with 28 kD glicoprotein (Gp28) from which it was purified by SDS-PAGE. The gel was immersed for 15 min in 6M urea and was subsequently placed in a dark chamber. P28 was found to consist of 2 bands (peptids), which were then separated from the gel and dialized against water, thus obtaining the pure Gp28.

2.4 Anti-*M. tuberculosis* hyperimmune serum

One New Zealand rabbit was immunized with the TPE. Pre-immune serum was obtained, then 100 μl of EPT with 100 μl of complete Freund's adjuvant were administered i.d. on days 1, 10 and 20 thus obtaining the anti-*M. tuberculosis* hyperimmune serum (HIS).

2.5 Polyclonal monospecific anti-p28 serum

One New Zealand rabbit was immunized with p28 following the same protocol as described above. Polyclonal monospecific anti-Gp28 serum was thus obtained.

2.6 TPE and Gp28 purity determination by Western blot

Three SDS-PAGE gels were prepared. Gel 1 was stained with Coomassie blue and contained the following samples in the corresponding wells: a) MW control; b) TPE; c) Seibert fraction 2 enriched with p28; d) pure p28. Gel 2 was used for Western blot incubated with HIS, and contained the following samples in the corresponding wells: e) TPE; f) pure p28. Gel 3 was used for Western blot incubated with anti-p28 monospecific polyclonal serum, and contained the following samples in the corresponding wells: g) TPE and h) pure p28. The Western blots were incubated with alkaline phosphatase labeled anti-rabbit antibody for 1 h at room temperature and were developed with NBT/BCIP.

2.7 Electrofocusing assay for p28

Two dimensional gel electrophoresis would indicate if Gp28 consist of 2 peptides or is formed by more peptides. [Rosenkrands, I., 2000]. It is realized in two parts:

Part 1. The electrofocusing gel contained: 1.1 g urea; 30 μl 10% 30%-acrylamide-(0.8% bis-acrylamide); 400 μl 10% NP-40; 80 μl pH 5-7 ampholyte; 20 μl pH 3.5-10 ampholyte; 5 μl 10% ammonium persulfate; 2 μl Temed; water q.s.p. 400 μl. The sample buffer contained: 2.85 g urea; 1ml 10% NP-40; 80 μl pH 5-7 ampholyte; 20 μl pH 3.5-10 ampholyte; 250 μl β-mercaptoethanol; water q.s.p. 5ml.

Gel pre run: a capillary test tube was filled with recently prepared gel, placed vertically, and when the gel had polymerized the sample was added at the top together with 5 mg of urea per each 10 μl of sample. The gel was run at 400 V, for 12 h, with the cathode buffer (20 mM NaOH) at the top and the anode buffer (10 mM H_3PO_4) at the bottom.

Part 2. A 10% acrylamide larger gel (30%-acrylamide-(0.8% bis-acrylamide)) was prepared. The gel was extracted from the capillary test tube and placed on the surface of larger previously polymerized gel. The gel was run at 100 V for 1 h. The gel was transferred to nitrocellulose paper and incubated with anti-Gp28 serum for 3 h. It was subsequently incubated with alkaline peroxidase-labeled anti-rabbit antibody raised in mouse for 1 h at room temperature, and subsequently developed with ortho-chloronaphthol.

2.8 IgG1 and IgG2 in immun anti-p28 sera by ELISA

Pre immune serum was obtained from 6 month-old Balb/c mice immunized i.p. with Gp28 diluted 1:1 with Freund's complete adjuvant. Immune sera were obtained at 1, 4, 8, 12 and 16 weeks after immunization. Immunoglobulin expression times were determined for Gp28 using ELISA.

Microtitration plates were sensitized by overnight incubation with Gp28 protein antigen in carbonate buffer. Thereafter, the plate was blocked with PBS.BSA for 1 h. The first antibody (mouse anti-Gp28 of 1, 4, 8, 12 or 16 weeks) was added to the wells, and incubated for 3 h.

This was followed by the second antibody (rabbit anti-IgG1 or anti-IgG2) to which alkaline phosphatase-labeled anti-rabbit serum was added. This was later developed with a solution of p-nitrophenyl phosphate disodium in diethanolamine buffer and the reaction blocked with 2N NaOH. The plate was read with an ELISA reader using a 405 nm filter.

2.9 Free mannose radicals in p28

A 10% acrylamide gel (30%-acrylamide-(0.8% bis-acrylamide)) with Gp28 protein in a well was run and transferred to nitrocellulose paper. The nitrocellulose paper was first incubated for 1 h with Concanavaline A-biotin diluted 5:1000 in PBS.BSA at room temperature and then for 1 h with streptavidin peroxidase at room temperarure It was developed with ortho-chloronaphthol.

2.10 p28 amino acid sequence

The Gp28 amino acid sequence was determined by Joe Gray of the Molecular Biology Unit, and Catherine Cookson of the Medical School at the University of Newcastle Upon Tyne. Results showed that Gp28 is a doublet consisting of two Gp28 kD bands containing amino acids A(M) – P(C) – K(Y) – V(E) –A (Y or L).

The predictive nucleotides for the 2 peptides were determined as:

A	P	K	V	A		M	C	Y	E	Y	L
GCC	CCC	AAG	GUC	GCC		AUG	UGC	UAC	GAG	UAC	CUG

These nucleotide sequences were not found in registered nucleotide banks and had not been previously identified.

2.11 Sodium periodate determination of sugar percentage in the anti-p28 antibody-recognizing epitope

ELISA method [Woodward, M. P., 1985]. The microtitration plate was sensitized with 5 μg Gp28 antigen per ml of PBS-BSA, placing 100 μl per well and incubating the plate overnight at 37°C. The plate was subsequently washed with 50 mM acetate buffer, pH 4.5. Recently prepared sodium periodate of 0.1, 1, 5 and 10 mM concentration in 50 mM acetate buffer, pH 4.5 was then added, each to a different well, and incubated in the darkness for 1 h at 37 °C. To block the aldehyde groups, the plate was incubated for 30 min at 37 °C with a recently prepared 1% glycine solution in PBS. The plate was washed with PBS-Tween 20 and immediately added 100 μl/well, with anti-Gp28 antibody and incubated for 1 h to 37°C, after which alkaline phosphate labeled anti-rabbit antibody was added and incubated for 1 h at 37°C. It was then developed with p-nitrophenyl phosphate disodium in diethanolamine buffer. The reaction was blocked with 2N NaOH and the plate was read in an ELISA reader at 405 nm.

2.12 Gene p28 from an *M. tuberculosis* library

The phage λgt11 was used to transfect lysogenic *E. coli*, strain Y1090hsdR, and construct a DNA expression library. Competent *E. coli* cell for λgt11 phage were incubated with 0.01 M

$MgSO_4$ for 1 h at 37°C. The phage carries the pMC9 plasmid that codes for the lac repressor, which, in turn, prevents the synthesis of fusion proteins potentially toxic for the β-galactosidase promoter. This plasmid carries the selective marker (amp^r). The HIS used was recognized by the strain H37Rv *Mycobacterium tuberculosis* library. The anti-p28 monospecific polyclonal serum was used to clone gene p28.

Titration of λgt11 phage. A 10 µl sample of the phage under study was added to a tube containing 1 ml dilution buffer, and a serial dilution was prepared in 10 tubes. The contens of each tube were next added to a tube containing Top agar a 50°C, and then transferred to a Petri dish containing Luria Bertani culture broth with ampicillin. Petri dishes were incubated at 37 °C for 24 h. Colonies were counted and those with 100 colonies were considered to contain $1x10^8$/ml λgt11 phage titre.

Titration of the M. tuberculosis library. A tube containing 1 ml of dilution buffer was added with 10 µl of λgt11 phage at $1x10^8$/ml titre and a serial dilution was prepared in 10 tubes. Each tube was added with 10 µl of the *M. tuberculosis* library. The contents of each tube were next added to a tube containing Top-agar at 50 °C and then transferred to a Petri dish containing Luria Bertani broth with ampicillin. They were then incubated at 37 °C for 24 h. Petri dishes with separate colonies were chosen and a nitrocellulose paper disc moistened with Isopropil-β-D-Thiogalactoside (IPTG) was placed in the dish marking its position and other nitrocellulose paper disc moistened with IPTG was placed in other dish marking its position These were then incubated for 1 h at 37 °C. The marked paper discs were then separated and one was incubated with HIS while the other was incubated with monoespecífic polyclonal anti-Gp28 for 3 h at 37°C, the two paper discs were incubated with alkaline peroxidase-labeled anti-rabbit anti-rabbit antibody for 1 h at room temperature.

Control. One tube containing 1 ml of dilution buffer was added with 10 µl of the studied phage. The contents of this tube were then added to a tube containing Top agar at 50°C and then transferred to a Petri dish containing Luria Bertini broth with amppicillin the dish was incubated for 24 h at 37°C. A nitrocellulose paper disk moistened with IPTG was placed in the dish marking its position, and it was then incubated for 1 h at 37°C. The disk was then separated from the Petri dish and incubated with HIS for 3 h at room temperature. Subsequently, it was incubated with alkaline peroxidase-labeled anti-rabbit serum for 1 h at room temperature and developed with NBT/BCIP. This served to prove that no protein from the *M. tuberculosis* library was recognized.

The Gp28 colonies were identified in the Petri dish and sown in Luria Bertani broth and ampicillin, incubated at 37 °C, 240 cycles/min for 20 h. Samples were then centrifuged, resuspended in lysis buffer centrifuged again and the supernatant was added with isopropanol to precipitate DNA, and incubated at 4 °C for 20 h. The samples were then centrifuged and the pellet was dissolved in 50 µl of Milli Q sterile water. A 2292 □g/µl concentration was determined by O.D. reading in a nanospectrophotometer; purity was established by the ratio of the values obtained at 260 nm = 52.697 and at 280 nm = 27.88 nm, which gave a value of 1.89 corresponding to DNA and of 0.11 corresponding to the protein. A 1% agarose gel with 1 µl/well of DNA diluted 1:10 was stained with ethidium bromide (10 µl/100 ml gel; obtained of 10mg/ml dilution) and run at 80 V for 50 min. Results were photographed.

2.13 Human Peripheral Blood Mononuclear Cells (PBMC)

PMBC were obtained from healthy subjects (PPD+, PPD-, with and without BCG vaccination), high risk subjects (staff from the Respiratory Disease Institute (Instituto de Enfermedades Respiratorias, INER)), and from tuberculosis patients (provided by INER) with the following characteristics:

Patient 1. Male, age 23, with untreated progressive tuberculosis; chest X-rays showing fibrosis in left lung and cavitary lesions in right lung.

Patient 2. Female, age 60, diabetic; chest X-rays showed unilateral and apical affliction .

Patient 3. Male, age 64, resistant to treatment with 6 years evolution.

Patient 4. Male, age 25, early infection; one brother died of tuberculosis at age 34, two sisters showed no infection.

Peripheral blood was obtained by venous puncture, subsequently it was treated with heparin (SIGMA, St. Louis Missouri, MO) at concentration of 10 U/ml of blood. An equal volume of RPMI medium was added. Samples of 8 ml of diluted blood were stratified with 4 ml of Ficoll-Hypaque. Tubes were centrifuged at 1500 rpm for 10 min to obtain PMBC and viability was determined with trypan blue.

2.14 T lymphocyte phenotype and antigen receptors

T lymphocytes were characterized by flow cytometry (FACScan, Becton Dickinson, San José, CA). PBMC were washed with RPMI medium and adjusted to $1x10^7$ cells/ml per patient. Next, 100 μl/tube were used to determine the following surface molecules: CD3, CD4, CD8, TCR αβ. The corresponding tube was added with 200 μl of first antibody OKT3, OKT4, OKT8, anti TCR αβ. All antibodies were raised in mouse. Tubes were incubated for 40 min at 4 ^oC in the darkness. Then they were washed 3 times with PBS for FACS, centrifuged, and the pellet resuspended in 500 μl 1% paraformaldehyde. Finally they were read by flow cytometry

3. Results

Purification of Gp28 carried out from the Seibert fraction was sucessful as illustrated by the single band obtained in a PAGE gel eluted with urea 6M and stained with Coomasie Blue (Figure 1). Molecular weight standards (band a) confirm the presence of Gp28 in TPE (band b), and in the enriched Seibert fraction (c). Purified Gp28 is contained in only one band, as shown in (d). TPE immuneblot is shown in (e) and when purified Gp28 is incubated with HIS or with anti-Gp28 serum (in bands f and g and h, respectively) only one band of 28 kD can be observed.

Electrofocusing of Gp28 indicated that it contains two peptides (see Figure 2). This was also confirmed by the N-terminal aminoacid sequences observed by Joe Gray from the Unit of Molecular Biology at Newcastle University.

ELISA analysis indicated that after 1, 4, 8, 12 and 16 weeks after Gp28 treatment, IgG2a is the predominant immunoglobulin detected in mice sera (see Figure 3).

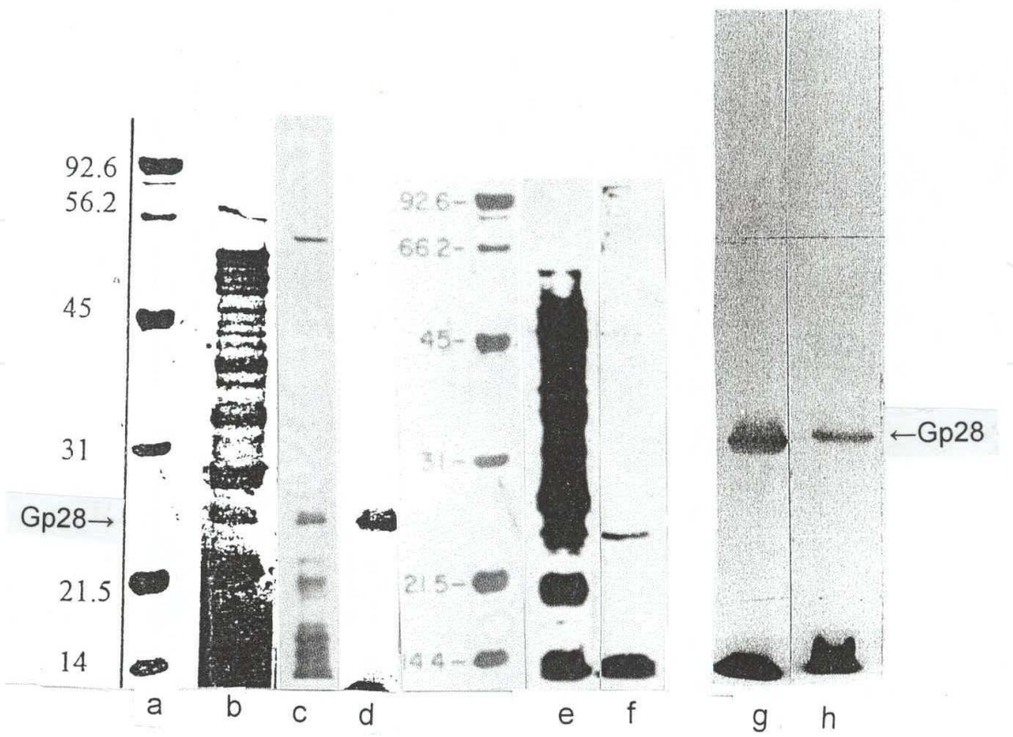


Fig. 1. Gels and immunoblots showing purification of the Gp28 protein isolated of *Mycobacterium tuberculosis* using TPE and Gp28 proteins. Gel stained with Coomassie blue contained the following sample, in **a**)MW; **b**) TPE; **C**) Seibert fraction 2; **d**) pure Gp28. Blot **e**) TPE; **f**) pure Gp28, In other blot **g**) TPE; **h**) pureGp28. Incubated with HIS or with monoespecific polyclonal anti-Gp28, showing pure Gp28

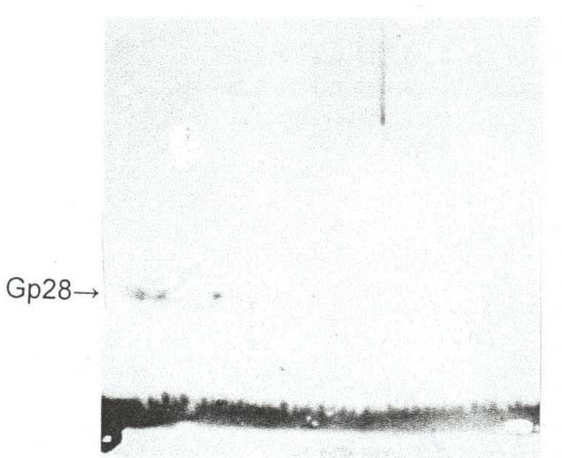


Fig. 2. Show than the protein is formed by only two peptides, which sequences was identified at the University of Newcastle Upon Tyne

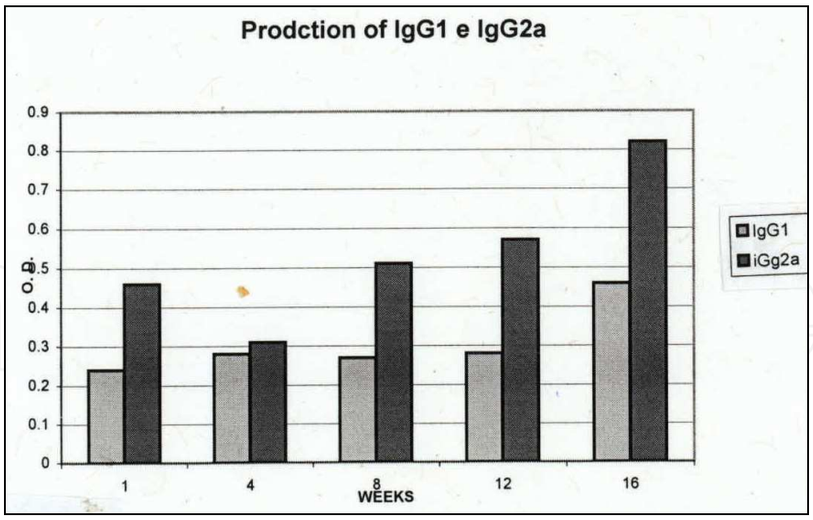


Fig. 3. Mice challenged with Gp28 showed that at 1, 4, 8, 12 and 16 weeks IgG2a predominate even after 16 weeks

The presence of free mannose residues is shown in the PAGE gel of Figure 4. Titration with NaIO3 indicates that Gp28 contains 43 % sugar residues, in the epitope that recognizes the antibody anti Gp28, and 57% peptide residues as shown in the ELISA assay in Figure 5.

Isolation of DNA coding for Gp28 was carried out using Luria Bertini plates blotted with nitrocellulose paper (Figure 6). Disc a presents staining attained when HIS prepared in our laboratory was incubated with a M. tuberculosis library. Disc b shows that HIS does not recognize any peptide when incubated only with λgt11 phage. When the policlonal monospecific anti-Gp28 is incubated with M. tuberculosis library only scattered staning is observed (Disc c). Figure 6d illustrates the electrophoresis of purified Gp28 DNA in an agarose gel that indicates the presence of 900 bp.

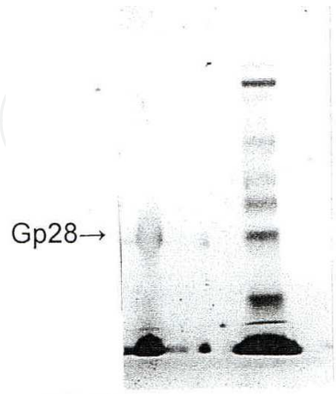


Fig. 4. 10 % acrilamida gel with Gp28 antigen in a well was run and transferred to nitrocellulose paper. This paper was incubated with Concanavaline A marked with Biotin and after it was incubated with streptavidin peroxidase and it is developed, showing that Gp28 is a glycoprotein, by the presence of free mannose radicals.

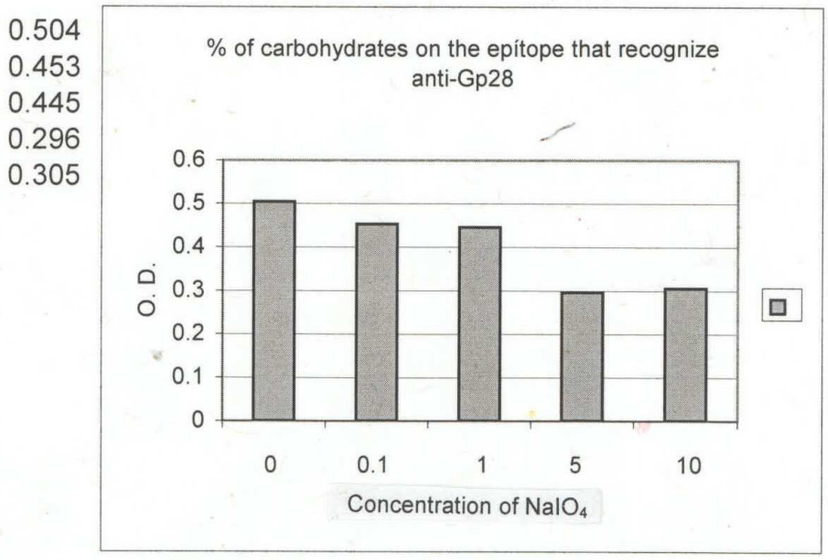


Fig. 5. The microtitration plate was sensitized with Gp28 antigens and incubating the plate overnight, The percentage of sugar in the epitope that recognize the anti-Gp28 was determined using sodium periodate of 0.1, 1, 5 and 10 Mm concentration in 50 mM acetate buffer pH 4.5. To block the aldehide groups with 1% glycine solution. Added with anti-Gp28. the Gp28 recognized the antigen a total of 43% of carbohydrates, leaving 57% of peptidic nature.

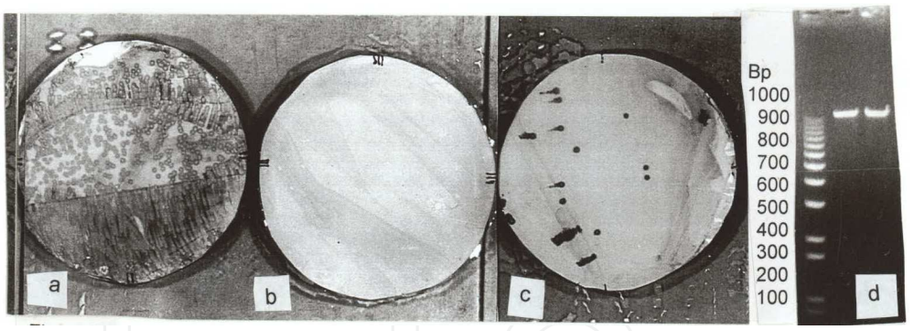


Fig. 6. The Gp28 DNA isolation process. **a**) the nitrocellulose paper incubated with HIS proved that *M. tuberculosis is recognized* in the library; **b**) no peptide of phago λgt11 was recognized in the nitrocellulose paper incubated with HIS; **c**) displays the nitrocellulose paper incubates with anti-Gp28 serum showing recognition of the Gp28; **d**) shows pure Gp28 DNA.

Incubation with pure Gp28 to induce specific cell lines in mononuclear cells obtained from venous human blood from non – vaccinated healthy subjects induced a significant increase of CD3+, CD4+ and TCRαβ+ (see Table 1). Vaccination of healthy subjects does not modify the CD3+, CD4+ nor CD8+ responses to Gp28, excepting for TCRαβ+ that is significantly higher when compared with the response observed in untreated patients with active TB: 92.5 – 94.7 versus 83.8 – 92.1 (IC 95%, $P<0.05$); this observation indicate which suggests that in active TB the response toGp28 appears to be significantly weakened. Otherwise the response to Gp28 of healthy subjects is not different from the one observed in untreated

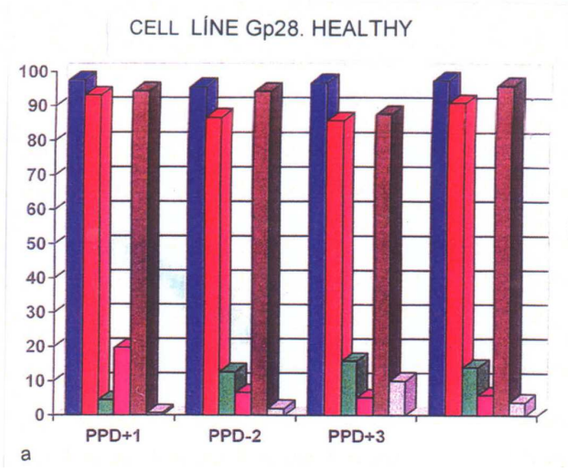
patients with active TB. The last line in Table 1 includes data from the literature for CD3+ (60 – 85), CD4+ (24 – 59) and CD8+ (18 – 48) that are consistent with the baseline values obtained in our laboratory in healthy subjects (Immunology Today, 1992). Considering the reduced number of patients analyzed, in Figure 7 are shown the individual responses to Gp28 observed in healthy subjects and untreated patients with active TB.

TABLE 1

Individual	CD3	CD4	CD8	CD4/CD8	TCRαβ
PPD+1	97.5	93.1	4.7	19.8	93.1
PPD-2	95.5	86.7	12.8	6.7	94.2
PPD+3	96.7	85.7	16.1	5.3	87.6
PPD-4	97.4	90.8	14.1	6.0	96.0

TABLE BASAL, NORMAL and CLASS

Individual	CD3	CD4	CD8	CD4/CD8	TCRαβ
PPD+3	51.1	34.6	23.0	1.5	52.3
PPD-4	59.5	50.4	18.3	2.7	62.7
Normal	73.0	44.0	33.0	1.2	95.0
Class	60-85	24-59	18-48		



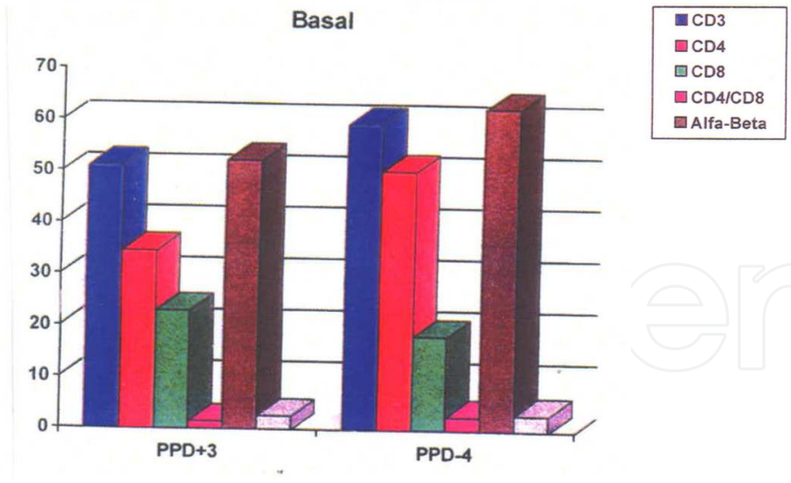


Fig. 7. and Table 1. *In vitro* production of CD3, CD4+, CD8+ and TCRαβ by the antigen specific cell lines induced with Gp28 protein obtained from healthy subjects high risk not vaccinated with BCG (PPD+1 and PPD-2) and vaccinated (PPD+3 and PPD-4), the phenotypic deviation were determined by FACS results confirmed that Gp28 induces proliferation of T helper (CD4+) by more than 90% in healthy.Table 1. bis. These results were compared with the same T cell type not treated with Gp28 of the same individuals, where they constituted approximately 50% of the total T lymphocytes.

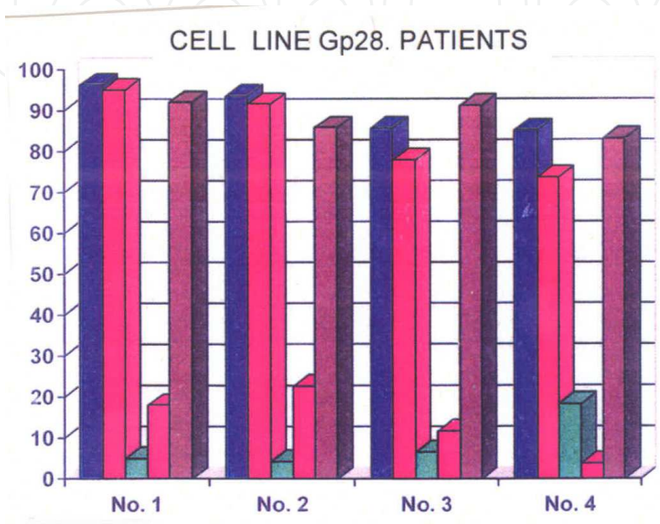


TABLE 2

Patient	CD3	CD4	CD8	CD4/CD8	TCRαβ
No. 1	96.3	94.9	5.1	18.0	92.0
No. 2	93.5	91.5	4.4	22.7	85.8
No. 3	85.7	77.8	6.7	11.6	91.1
No. 4	85.1	73.5	18.4	3.9	83.1

Fig. 8. and Table 2. In the studied tuberculosis patients CD4+ percentage increased by 90% in patients 1 and 2 and 73% in patients 3 and 4, these results are probably related with the severity of TB in these patients.

	HS		TB
Value for cell cluster density	Baseline values of non-vaccinated subjects (n=2)	Challenged with Gp28 (n=4)	Challenged with Gp28 (n=4)
CD3+	55.3 (47.1 – 63.5)*	96.8 (95.8 – 97.6)	90.1 (84.6 – 95.6)
CD4+	42.5 (27.1 – 57.9)*	89.1 (85.6 – 92.4)	84.4 (74.2 – 94.5)
CD8+	20.7 (16.0 – 25.2)	11.9 (7.0 – 16.8)	8.7 (2.2 – 15.0)
TCRαβ+	57.5 (47.3 - 67.6)*	92.7 (89.1 – 96.2)	88.0 (83.8 – 92.1)
CD4+/CD8+	2.1 (0.9 – 3.2)	9.4 (2.6 – 16.2)	14.1 (6.0 – 22.0)

Reference baseline values from (Immunology Today, 1992) for CD3+ (60 – 85), CD4+ (24 – 59), and CD8+ (18 – 48) are consistent with our findings. Data shown are mean value and confidence intervals estimated for a P value of 0.05 (IC 95%). *Indicates significant difference (P<0.05) when comparing baseline values of non-vaccinated HS with vaccinated or non-vaccinated HS challenged with Gp28. There is no significant difference between HS and TB when challenged with Gp28.

Statistic Values: confidence intervals P value of 0.05 (IC 95%).*significant diference P<0.05

Table 3. Effect of Gp28 on phenotype deviation of human peripheral monouclear cells obtained from venous blood of healthy subjects (HS) and from untreated patients with active tuberculosis (TB).

4. Conclusions

In view of the dissimilar protection conferred by the BCG vaccine, research has focused on the identification of immuneprotective antigens. This necessity has been recently magnified the increase in rates of TB, the appearance of bacilli resistant to multiple antituberculous drugs and the rise in the frequency of immunosupressor diseases [Olobo, JO., 2001]. The strategy followed in this study was first to purify the Gp28 antigen of a virulent strain of *Mycobacterium tuberculosis* from the culture medium and to examine its biochemical characteristics. The presence of the mannose radicals [Ehlers, MR., 1998. Heldwein, KA., 2002] was determined, which are identified by the complement receptors CR1, CR3 and CR5 of macrophages, inducing non-opsonic TB bacillus phagocytosis. Sugar and peptide porcentages in the epitope recognized by anti-Gp28 serum were investigated, and IgG2a was identified as the predominant immunoglobulin. The protein was found to consist of a peptide doublet. The N-terminal amino acids of the two peptides were determined as well as the predictive nucleotides, which had not been identified before. The interest in Gp28 was stimulated by its recognized capacity to induce IL2 and IFNγ and are know to be immunocompetent. Evidence has been obtained that protective immunity in tuberculosis is related to CD4+, CD8+ and TCRαβ [Zhan, M., 1995. Lazarevic, V., 2002] In the present experiment peripheral mononuclear blood cells (PMBC)from healthy individuals and TB patients were incubated with Gp28 to obtain cell lines and their phenotype and antigen receptors were determined by FACS. Results confirmed that Gp28 induces proliferation of T

helper cells (CD4+) by more than 90% in healthy individuals, and TCRαβ also increased by more than 90%, with the single exception of one individual (PPD+3) who showed an 87% increase. These results were compared with the same T cell type not treated with Gp28 of the same individuals, where they constituted approximately 50% of the total T lymphocytes, In the studied tuberculosis patients CD4+ percentage increased by 90% in patients 1 and 2 and by 73% in patients 3 and 4. As to TCRαβ, these increased by 90% in patients 1 and 3 and by 83% in patients 2 and 4, these results are probably related with the severity of TB in these patients. Protein Gp28 exhibits an epitope capable of inducing the T response more intensely in healthy PPD+ than in PPD- individuals the application of BCG surely stimulated the adaptive immune response against the TB bacillus in PPD+ individuals than in PPD- individuals, the application of BCG surely stimulated the adaptative immune response against the TB bacillus in PPD+ individuals.

5. Acknowledgment

I want to thank Ing. M. en C. Mario Farías Elinos for excellent adviser in computation

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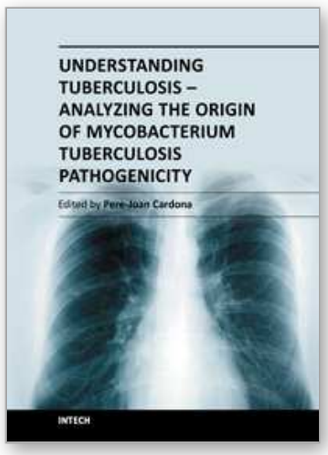
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Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity

Edited by Dr. Pere-Joan Cardona

ISBN 978-953-307-942-4
Hard cover, 560 pages
Publisher InTech
Published online 24, February, 2012
Published in print edition February, 2012

Mycobacterium tuberculosis in an attempt to understand the extent to which the bacilli has adapted itself to the host and to its final target. On the other hand, there is a section in which other specialists discuss how to manipulate this immune response to obtain innovative prophylactic and therapeutic approaches to truncate the intimal co-evolution between Mycobacterium tuberculosis and the Homo sapiens.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Elinos-Báez Carmen Martha and Ramírez González (2012). Biochemical and Immunological Characterization of the Mycobacterium tuberculosis 28 kD Protein, Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-942-4, InTech, Available from: http://www.intechopen.com/books/understanding-tuberculosis-analyzing-the-origin-of-mycobacterium-tuberculosis-pathogenicity/biochemical-and-immunological-characterization-of-the-mycobacterium-tuberculosis-28-kd-protein

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