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Active tuberculosis patients have high levels of IgA anti-alpha-crystallin and isocitrate lyase proteins

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SUMMARY

SETTING: Mexico City, Mexico.

OBJECTIVE: To identify proteins synthetised by *Mycobacterium tuberculosis* in hypoxic culture, which resemble more closely a granuloma environment than aerobic culture, and to determine if they are recognised by antibodies from patients with active pulmonary tuberculosis (PTB).

DESIGN: Soluble extracts from *M. tuberculosis* H37Rv cultured under aerobic or hypoxic conditions were analysed using two-dimensional polyacrylamide gel electrophoresis, and proteins over-expressed under hypoxia were identified by mass spectrometry. The presence of immunoglobulin (Ig) G, IgA and IgM antibodies against these proteins was determined in the serum of 42 patients with active PTB and 42 healthy controls.

RESULTS: We selected three *M. tuberculosis* H37Rv proteins (alpha-crystallin protein [Acr, Rv2031c], universal stress protein Rv2623 and isocitrate lyase [ICL, RV0467]) that were over-expressed under hypoxia. Titres of anti-Acr and anti-ICL IgA antibodies were higher in patients than in healthy controls, with an area under the receiver operating characteristic curve of 0.71 for anti-ICL IgA antibodies.

CONCLUSION: ICL could be used in combination with other *M. tuberculosis* antigens to improve the sensitivity and specificity of current serological TB diagnostic methods.

KEY WORDS: serological diagnostic methods; hypoxia; 2D-PAGE/MS

TUBERCULOSIS (TB), A DISEASE caused by *Mycobacterium tuberculosis*, remains a worldwide health problem. TB is mainly a lung disease; most persons infected with *M. tuberculosis* control the infection but do not eliminate the mycobacteria, which persist inside granulomas without causing overt disease (latent tuberculous infection [LTBI]), and may eventually cause active disease.[1] TB is diagnosed according to classical clinical presentation, chest X-ray and the microscopic detection of acid-fast bacilli (AFB) in sputum samples, which is only useful in the late stages of active pulmonary TB (PTB), when sufficient bacilli are expelled from the lungs, and does not detect paucibacillary patients or patients with extra-pulmonary TB. TB can also be diagnosed by mycobacteria culture of the sputum using nucleic acid amplification, and by the detection of interferon gamma (IFN-γ) production by peripheral blood mononuclear cells in response to mycobacterial antigens. These methods are, however, more expensive and some of them require specialised training and equipment.[1]

Serological diagnostic methods are rapid, inexpensive and easy to perform. Several serological diagnostic methods for TB have been proposed and evaluated, but none has been found to have sufficient sensitivity and specificity to be used reliably in clinical settings. Many of these diagnostic methods target antigens that are produced in high quantities in culture media, or antigens that are immunodominant in laboratory animals immunised with extracts from cultured mycobacteria. These antigens (Hsp65

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[Rv0440], 38-kDa lipoprotein [Rv0934], 19-kDa lipoprotein [Rv3763], culture filtrate protein 10 [CFP-10; Rv3874], early secretory antigenic target 6 [ESAT-6; Rv3875], Ag85A [Rv3804c] and others) have been evaluated, alone or in combination, as possible targets for serological diagnostic methods for TB,[2] but they may not be the predominant proteins that *M. tuberculosis* produces during human infection.

Granulomas, the defining pathological characteristic of PTB, are organised structures formed by neutrophils, monocytes, macrophages, giant multinucleated cells, foamy macrophages, epithelioid macrophages, lymphocytes, blood vessels and an outer fibrous layer;[3] the macrophage-rich centre is hypoxic, acidic and nutrient-depleted.[4] In the granuloma, the mycobacteria enter a stationary growth phase and eventually become non-replicating bacilli; these bacteria, also known as latent or persistent bacilli, can resume growth under favourable ambient conditions.[5] Several in vitro latency models induced by hypoxia,[6] nutrient depletion,[7] low pH[8] and nitric oxide[9] have been used to characterise latent bacilli. Hypoxia induces the differential expression of several genes that may protect the mycobacteria from the deleterious effects of the granuloma environment and promote mycobacterial survival for prolonged periods of time.[10]

Yuan et al. identified 16 kDa alpha-crystallin protein (Acr, Rv2031c) as the predominant protein in *M. tuberculosis* stationary-phase cultures,[11] while Starck et al. reported that Acr and several mycobacterial proteins are increased during hypoxia.[12] The proteins synthetised by *M. tuberculosis* under hypoxia could be used, in combination with other antigens such as ESAT-6 and CFP-10, to increase the sensitivity and specificity of serological diagnostic methods for TB. In this study, we identified three *M. tuberculosis* proteins, Acr, universal stress protein Rv2623 and isocitrate lyase (ICL) RV0467, which are produced by *M. tuberculosis* under hypoxic conditions, and we tested the hypothesis that patients with active PTB would have antibodies against these hypoxia-expressed *M. tuberculosis* proteins. We found that Acr and ICL were recognised by antibodies present in the serum of patients with active PTB, and suggest that ICL could be used to improve the sensitivity and specificity of current serological TB diagnostic methods.

MATERIALS AND METHODS

Patient selection criteria

Forty-two patients aged 21–67 years (36% females, 64% males) with a first episode of active PTB were recruited from the General Hospital of Mexico, Mexico City, Mexico. Active PTB was diagnosed based on clinical presentation of the disease and chest X-ray; all patients were AFB sputum-positive. Six of the patients (14%) were sputum culture-negative, but all of them responded to anti-tuberculosis treatment consisting of isoniazid, rifampicin, pyrazinamide and ethambutol.[13] Pregnant patients, patients who had undergone recent (<4 weeks) major surgery and patients with immunosuppressive treatment, immunodeficiency, human immunodeficiency virus infection, cancer, diabetes mellitus or auto-immune disease were not included in the study. Sixty-two healthy controls from the same endemic area as the TB patients aged 20–57 years (66% females, 34% males) were also included in this study; controls had no clinical evidence of active TB and no close contact with active TB patients. All patients and controls had received bacille Calmette-Guérin vaccination at birth.[14] IFN-γ release assay (IGRA; QuantiFERON®-TB Gold, Qiagen, Valencia, CA, USA) was performed among 20 of the healthy controls to detect LTBI.

All serum samples were obtained after informed consent had been given. The study was approved by the Ethics and Research Committee of the General Hospital of Mexico, Mexico City (DIC/07/406/03/007).

Mycobacterial culture

M. tuberculosis H37Rv was cultured in Dubos medium supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) at 37°C and 200 rpm for 7–9 days to an optical density 600 (OD_{600}) of 0.4–0.45 (exponential growth phase). To induce hypoxia, an exponential culture was harvested by centrifugation, resuspended in Dubos medium with 10% OADC and placed in sealed flasks, with an initial volumetric ratio of air to medium of 1:2. A parallel culture supplemented with methylene blue (1.5 μg/ml) was used as an indicator of oxygen depletion. The cultures were incubated at 37°C with a magnetic stirrer for 15 days, at which time the methylene blue indicator was completely reduced and the mycobacteria were considered to be in the non-replicating persistent phase 2 (NRP2) of the Wayne and Hayes model.[6]

Preparation and analysis of M. tuberculosis *soluble extract*

Mycobacteria pellets were washed and resuspended in phosphate buffer saline (PBS) with cOmplete™ Protease Inhibitor Cocktail (Roche Diagnostics, Basel, Switzerland) at a concentration of 500 μg per 500 μl. This suspension was disrupted with sterile glass beads (150–212 μm), with 8 cycles (6.5 m/s, 15 s) using a FastPrep BIO101 (Thermo Savant, Carlsbad, CA, USA), and centrifuged at 10 000 x *g* at 4°C for 5 min to obtain mycobacteria-free supernatants (MTSE). MTSE protein concentration was determined using the Quick Start Bradford Dye Reagent

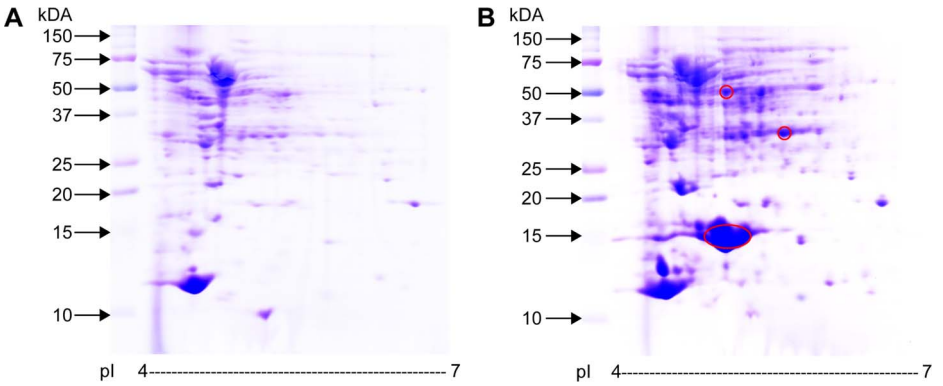

Figure 1 The proteins produced by *Mycobacterium tuberculosis* in hypoxic culture are different from those produced in aerobic culture. The panels show soluble extracts obtained from **A)** *M. tuberculosis* H37Rv cultured at 37°C under aerobic conditions (exponential growth), and **B)** *M. tuberculosis* H37Rv cultured under hypoxia (Wayne and Hayes non-replicating persistent phase 2). *M. tuberculosis* soluble extract was separated using two-dimensional polyacrylamide gel electrophoresis with an isoelectric focusing pH range of 4–7 and a 12% sodium dodecyl sulfate polyacrylamide gel, and stained using Coomassie Brilliant Blue R-250. The figure is representative of 10 independent experiments; equal amounts of proteins were loaded in all gels. Spot numbers in Figure 1B correspond to those in Tables 1 and 2. This image can be viewed online in colour at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/00000012/art00021

(BioRad, Hercules, CA, USA). MTSE were separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE);[12] images were acquired in a G-Box scanner with Gene Snap Software v. 6.08 (Syngene, Cambridge, UK). Selected proteins were identified with a liquid chromatography-mass spectrometry system (Accela fluid flow micro-chromatograph, LTQ Orbitrap Velos, Thermo Fisher Scientific, Waltham, MA, USA). The spectrometric data were verified against the *M. tuberculosis* protein data bank using Proteome Discoverer software (v. 1.3, Thermo Fisher Scientific).

Detection of serum antibodies that recognize M. tuberculosis *soluble extract proteins by enzyme-linked immunosorbent assay*

Recombinant *M. tuberculosis* H37Rv alpha-crystallin protein (Acr)[15] and ICL (donated by J C Sacchettini, Department of Chemistry, Texas A&M University, College Station, TX, USA) were purified using fast protein liquid chromatography (FPLC) in a Superose 12/10/300 molecular exclusion column (Amersham Biosciences, Uppsala, Sweden). Each well of a 96-well plate was coated with 0.1 µg of Acr or 3.2 µg of ICL in carbonate buffer, and the plate was left at 4°C overnight. The wells were washed five times with PBS and 10 times with PBS 0.05% Tween 20 (PBS-T) and blocked with 200 µl PBS with 3% skimmed milk for 1 h. After washing, 100 µl of serum, diluted 1:4000 in PBS with 3% skimmed milk, were added to each well and the plate was incubated for 2 h at room temperature. After washing, horseradish peroxidase (HRP) conjugated secondary antibodies were added (anti-human immunoglobulin [Ig] G antibody 1:2000, Jackson ImmunoResearch Laboratories, West Grove, PA, USA; anti-human IgA antibody 1:500 and anti-human IgM antibody 1:500, Thermo Fisher Scientific), and the plate was incubated for 1 h at room temperature. After washing, 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide were added, and after 30 min in the dark the absorbance at 450 and 570 nm was measured using a Multiskan EX Microplate Reader (Thermo Fisher Scientific).

Statistical analysis

Serum antibody levels are depicted as 'OD 450 nm', which represents ($A_{450–570nm}$ of the sample)–($A_{450–570nm}$ of the blank). Student's *t*-test and receiver-operator characteristic (ROC) curves were calculated using Prism v. 6.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

M. tuberculosis *changes its proteome when cultured under hypoxia*

Soluble extracts from *M. tuberculosis* H37Rv cultured under aerobic (exponential growth) or hypoxic conditions (NRP2) were separated using 2D-PAGE. Many proteins were expressed in both conditions, but some proteins with isoelectric points of between 5 and 7 and molecular weights of between 10 and 100 kDa were over-expressed or synthesised de novo in the hypoxic cultures (Figure 1). We selected three proteins that were consistently over-expressed in the hypoxic cultures but not in the aerobic cultures (circles in Figure 1B); the observed molecular weights and isoelectric points of these proteins are shown in Table 1. The proteins were excised from the gel and

Table 1 Identification of three *Mycobacterium tuberculosis* H37Rv proteins expressed under hypoxia and recognised by the serum of tuberculosis patients

Spot	Molecular weight kDa (observed/calculated*)	Isoelectric point pI (observed/calculated*)	Organism†	Name of protein†	Name of gene†	Name of ordered locus†
1	16/16.227	4.75/5.00	*M. tuberculosis* (strain ATCC 25618/H37Rv)	Alpha-crystallin protein	*hsp*X	Rv2031c
2	31.6/31.652	5.55/5.45	*M. tuberculosis* (strain ATCC 25618/H37Rv)	Universal stress protein Rv2623	*Rv*2623	Rv2623
3	47.1/47.087	4.79/5.03	*M. tuberculosis* (strain ATCC 25618/H37Rv)	Isocitrate lyase	*icl*	Rv0467

***** Compute pI/Mw tool, ExPASy Bioinformatics Resource Portal.[16]
† Universal Protein Resource (UniProt).[17]
ATCC = American Type Culture Collection.

analysed using mass spectrometry. The spectrometric data were verified against the *M. tuberculosis* protein data bank using the Proteome Discoverer software; this strategy identified the candidate proteins as *M. tuberculosis* H37Rv Acr, universal stress protein Rv2623 (USP Rv2623) and ICL (Tables 1 and 2).

Patients with active PTB have antibodies against M. tuberculosis *proteins produced under hypoxia*

As the proteins expressed by *M. tuberculosis* under hypoxic cultures differed from those expressed under aerobic cultures, we hypothesised that patients with active PTB would mainly have antibodies against the *M. tuberculosis* proteins expressed under hypoxia. To test this hypothesis, we measured the amounts of IgG, IgA and IgM antibodies specific for recombinant Acr or ICL in the serum of patients with active PTB and in that of healthy controls. We found that TB patients had higher titres of anti-Acr and anti-ICL IgA antibodies (Figure 2) than healthy controls. No differences were found in the titres of anti-Acr and anti-ICL IgG and IgM antibodies. The highest area under the ROC curve (AUC) was obtained for the anti-ICL IgA antibodies (0.71), while the AUC for the anti-Acr IgG, IgA and IgM antibodies and the anti-ICL IgG and IgM antibodies were all <0.53 (Figure 3). IGRA-negative and IGRA-positive healthy controls had no difference in titres of anti-Acr and anti-ICL IgA antibodies (Figure 4). For anti-ICL IgA antibodies, a cut-off value of OD 450 nm > 0.5570 gives a sensitivity of 69% (95% confidence interval [CI] 53–82) and a specificity of 62% (95%CI 46–76).

DISCUSSION

Serological diagnostic methods are valuable tools because they are rapid, inexpensive and easy to perform. However, the success of these methods

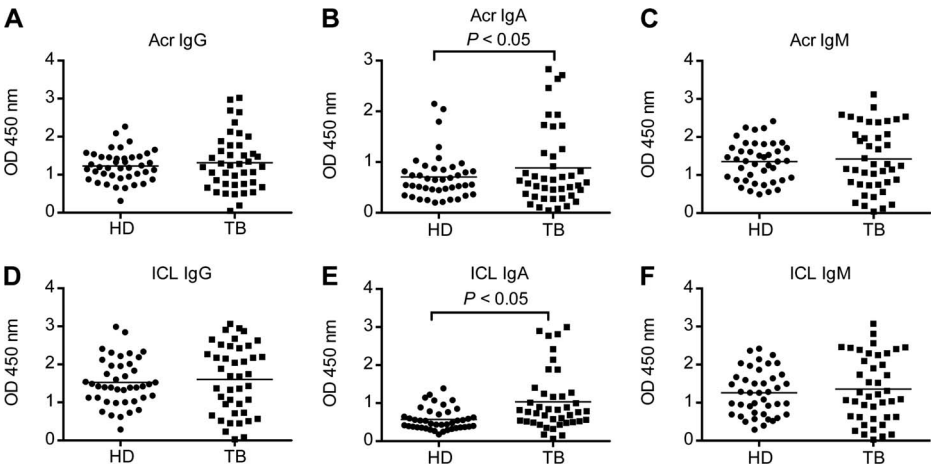

Figure 2 TB patients have higher titres of anti-Acr and anti-ICL IgA antibodies than healthy controls. The serum of 42 patients with active pulmonary TB aged 21–67 years (36% females, 64% males) and 42 healthy controls aged 20–27 years (62% females, 38% males) were analysed using enzyme-linked immunosorbent assay, **A–C)** with fast protein liquid chromatography-purified recombinant *Mycobacterium tuberculosis* H37Rv Acr; and **D–F)** *M. tuberculosis* H37Rv ICL coating the plates; and **A and D)** anti-human IgG, **B and E)** IgA or **C and F)** IgM as secondary antibodies. Each point in the graphs (OD 450 nm) represents ($A_{450–570nm}$ of the sample)–($A_{450–570nm}$ of the blank). Data were analysed using Student's *t*-test, and significance was set at $P < 0.05$. OD = optical density; Acr = alpha-crystallin protein; Ig = immunoglobulin; HD = healthy controls; TB = tuberculosis; ICL = isocitrate lyase.

Table 2 MS-generated peptides for *Mycobacterium tuberculosis* protein identification

Spot	Name of protein	Peptides generated by MS (modifications)
1	Alpha-crystallin protein	Coverage 79.17%; 10 peptides: GILTVSVAVSEGKPTEK TVSLPVGADEDDIKATYDK SEFAYGSFVR AELPGVDPDKDVDIMVR; M15 (oxidation) TVSLPVGADEDDIK TEQKDFDGR DGQLTIK LEDEMKEGR; M5 (oxidation) SLFPEFSELFAAFPSFAGLRPTFDTR AELPGVDPDKDVDIMVR
2	Universal stress protein Rv2623	Coverage 46.13%; 11 peptides: AGPPTVHSEIVPAAAVPTLVDMSK; M22 (oxidation) AGPPTVHSEIVPAAAVPTLVDMSK GGYAGMLVGSVGETVAQLAR; M6 (oxidation) DAVLMVVGCLGSGR; M5 (oxidation); C9 (carbamidomethyl) LLGSVSSGLLR YPNVAITR MSSGNSSLGIIVGIDDSPAAQVAVR SEEAQLVVVGSR HLIDDALK VVEQASLR TPVIVAR
3	Isocitrate lyase	Coverage 83.64%; 31 peptides: ALIAAGVAGSHWEDQLASEK IATTVDPNSSTTALTGSTEEGQFH TYSAEDVVALQGSVVEEHTLAR TDAEAATLITSDVDER ALIAAGVAGSHWEDQLASEKK AIYLSGWQVAGDANLSGHTYPDQSLYPANSVPQVVR AEYPDQMLAYNCSPSFNWK; C12 (carbamidomethyl) AEYPDQMLAYNCSPSFNWK; M7 (oxidation); C12 (carbamidomethyl) AYAPFADLIWMETGTPDLEAAR AYAPFADLIWMETGTPDLEAAR; M11 (oxidation) LAADVADVPTVVIAR SAEQIQQEWDTNPR TDAEAATLITSDVDERDQPFITGER HLDDATIAK IEGDTSVENWLAPIVADGEAGFGGALNVYELQK DQPFITGER NGIEPCIAR; C6 (carbamidomethyl) TREGFYR VLIPTQQHIR EVGAGYFDR CGHLGGK; C1 (carbamidomethyl) GAEVLWEQLHDLEWVNALGALTGNMAVQQVR; M25 (oxidation) ELAAMGFK ELAAMGFK; M5 (oxidation) TKNGIEPCIAR; C8 (carbamidomethyl) RINNALQR EFAAEER TLTSAR QFSEAVK KHLDDATIAK GYTATK

MS = mass spectrometry.

depends on the identification of the most relevant antigenic targets. Most of the serological diagnostic methods that have been evaluated for TB target antigens are produced in high quantities when *M. tuberculosis* is cultured under aerobic conditions, but the proteins that are over-expressed when *M. tuberculosis* is cultured under hypoxia may be more representative of the proteins that the mycobacteria produce in vivo inside the granuloma.[18,19] In this study, we identified three *M. tuberculosis* H37Rv proteins (Acr, USP Rv2623 and ICL) that are produced under hypoxia. Acr is overexpressed by *M. tuberculosis* during stationary growth and hypoxia and inside macrophages.[11,12] USP Rv2623 regulates *M. tuberculosis* latency and attenuates mycobacterial growth in vitro,[20] while ICL is involved in mycobacterial growth regulation, virulence and persistence inside the host;[21] both proteins

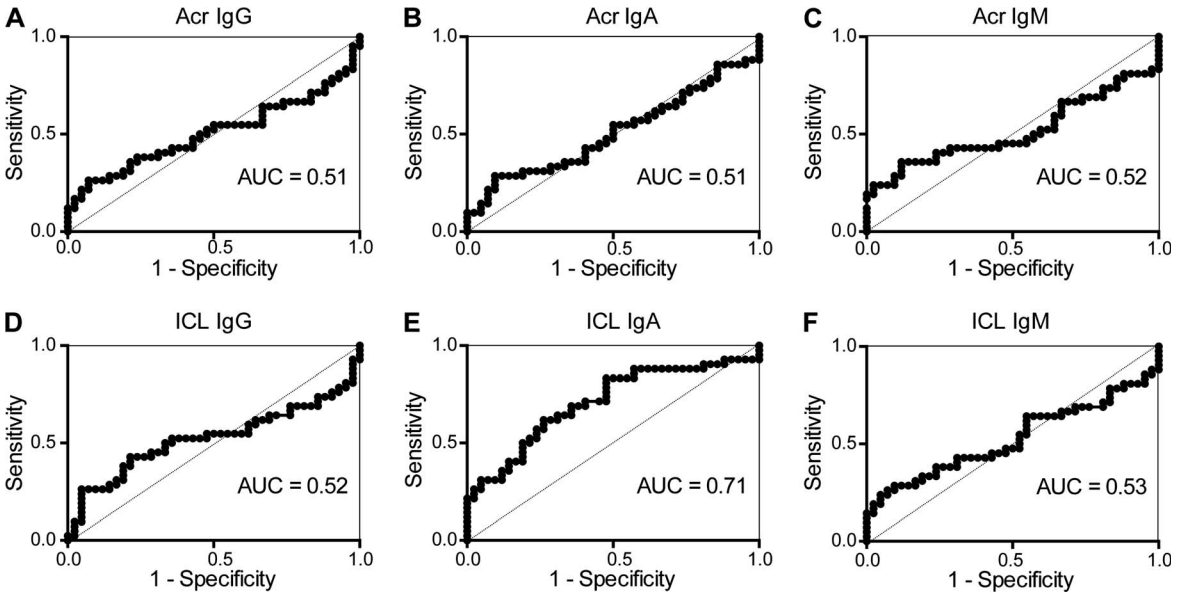

Figure 3 ROC curve analysis of the anti-Acr and anti-ICL IgG, IgA and IgM antibodies. The serum of 42 patients with active pulmonary TB and 42 healthy controls were analysed using enzyme-linked immunosorbent assay, with **A–C)** fast protein liquid chromatography-purified recombinant *M. tuberculosis* H37Rv Acr; and **D–F)** *M. tuberculosis* H37Rv ICL coating the plates; and **A and D)** anti-human IgG, **B and E)** IgA or **C and F)** IgM as secondary antibodies. The AUC is indicated in each graph. For anti-ICL IgA antibodies, a cut-off value of OD 450 nm > 0.5570 gives a sensitivity of 69% (95%CI 53–82) and a specificity of 62% (95%CI 46–76). ICL = isocitrate lyase; Ig = immunoglobulin; AUC = area under the ROC curve; Acr = alpha-crystallin protein; ROC = receiver operating characteristic; OD = optical density; CI = confidence interval.

are strongly induced when *M. tuberculosis* is subjected to hypoxia.[20,21]

We detected the presence of anti-Acr and anti-ICL IgG, IgA and IgM antibodies in the serum of patients with active PTB. The titres of IgA antibodies were significantly higher in TB patients than in healthy controls, which supports the notion that the mycobacteria produce high amounts of these proteins in

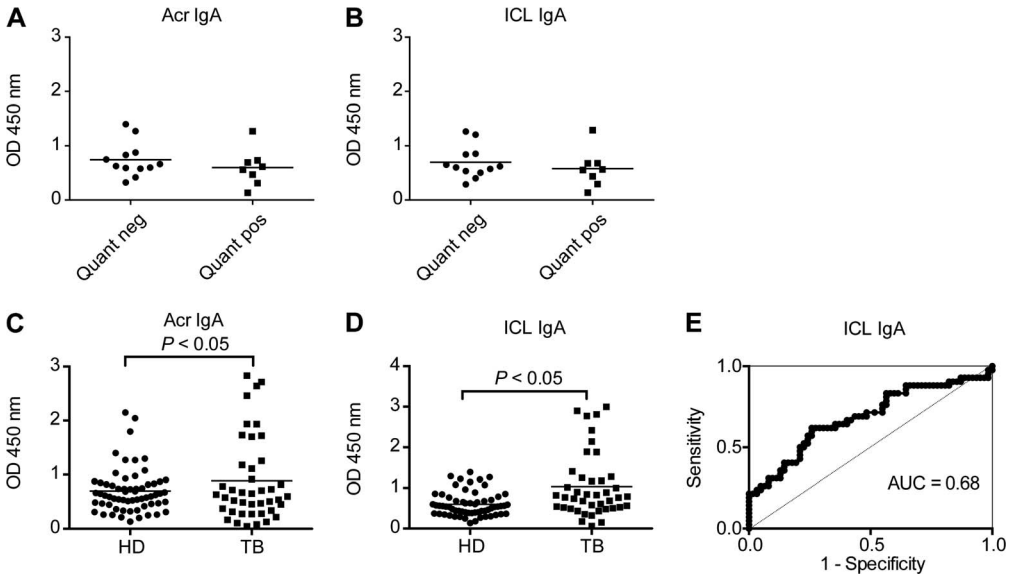

Figure 4 Latent tuberculous infection has no effect on anti-Acr and anti-ICL IgA antibodies. Twenty healthy controls aged 29–57 years (75% females, 25% males) were tested with an IGRA (QuantiFERON®-TB Gold). The serum of 12 IGRA-negative and 8 IGRA-positive healthy controls was analysed using enzyme-linked immunosorbent assay, with **A)** fast protein liquid chromatography-purified recombinant *M. tuberculosis* H37Rv Acr and **B)** *M. tuberculosis* H37Rv ICL coating the plates, and anti-human IgA as secondary antibody. Data were analysed using Student's *t*-test. The data of these 20 healthy controls were analysed together with the data of the 42 healthy controls and the 42 patients with active pulmonary TB depicted in Figures 2 and 3, for **C)** anti-ACR IgA antibodies, **D)** anti-ICL IgA antibodies (Student's *t*-test) and **E)** anti-ICL IgA antibodies (AUC). OD = optical density; Acr = alpha-crystallin protein; Ig = immunoglobulin; ICL = isocitrate lyase; Quant = QuantiFERON®-TB Gold; HD = healthy controls; TB = tuberculosis; IGRA = interferon gamma release assay.

the lungs of patients. We found that, in both IGRA-negative and IGRA-positive healthy controls, some individuals had detectable anti-Acr and anti-ICL antibodies, but the anti-Acr and anti-ICL IgA antibody levels were not significantly different between these two groups; active TB patients have significantly higher anti-Acr and anti-ICL IgA antibody titres than both groups. IGRA-positive healthy controls are currently defined as having 'LTBI'; however, LTBI encompasses a wide spectrum of conditions: at one end are patients without clinical disease but with actively replicating mycobacteria;[22,23] at the opposite end are individuals who have successfully cleared the infection and have T-cell memory.[22,24] In the former case, mycobacterial antigens can percolate from the granuloma, reach the general circulation and drive antibody formation;[22] it is now accepted that the granuloma is not a static structure, but a dynamic interaction between the pathogen and the host.[25,26] In our study, the IGRA-negative healthy controls with detectable anti-Acr and anti-ICL antibody titres could represent individuals that have successfully cleared the infection and have antibodies against *M. tuberculosis* antigens, but not T-cell memory.[27] Our results indicate that the titres of anti-ICL IgA antibodies have high sensitivity and specificity (AUC 0.71) and could be used to distinguish patients with active PTB from healthy controls, probably not as a standalone test, but in combination with other antigens: ICL could be used to increase the sensitivity and specificity of other serological diagnostic methods for TB. Alternatively, anti-ICL IgA antibodies could be useful to improve the diagnosis in patients with clinical symptoms but without detectable AFB in their sputum samples.

Several authors have reported the advantages of antigen combinations over single antigens in serological diagnostic methods for TB. Uma et al. found IgG, IgA and IgM antibodies against *M. tuberculosis* 38-kDa lipoprotein in patients with active PTB, with sensitivities of 61% for IgG, 30% for IgA and 10% for IgM antibodies; the combination of the three antibody classes increased the sensitivity to 71.4%.[28] The detection of IgG antibodies against a combination of Acr, TB16.3 (Rv2185c), 3-dehydroquinate dehydratase (Rv2537c), 30S ribosomal protein S15 (Rv2785c) and lipoprotein Rv3354 had a sensitivity of 76% and a specificity of 96% for the detection of patients with active TB.[29] The antibody response to *M. tuberculosis* antigens varies considerably between individuals,[30] indicating that a mixture of several antigens would be a more efficient way to cover the heterogeneity of this antibody response and to increase the sensitivity of serological diagnostic methods for TB.

Human TB lesions are highly hypoxic,[31] and other studies have reported that antibodies against proteins that *M. tuberculosis* expresses under hypoxia, such as Rv2041c[19] and Acr,[32] are present in patients with active PTB. Wolfe et al. reported that *M. tuberculosis* ICL, along with members of the DosR regulon and other proteins involved in energy metabolism and lipid biosynthesis, are overexpressed when *M. tuberculosis* is cultured under hypoxia,[33] and, as there are no ICL orthologues in mammals, ICL has been proposed as a potential target for the development of new drugs for the treatment of TB.[33,34] To our knowledge, ICL has not been previously reported as a possible serodiagnostic target for PTB. Our results support the notion that hypoxia is an important condition for the further development of serological TB diagnostic methods.

CONCLUSION

Acr and ICL are overexpressed by *M. tuberculosis* during hypoxia, and are recognised by serum antibodies from patients with active PTB. The titres of anti-ICL IgA antibodies could be used to discriminate between TB patients and healthy controls, in combination with other *M. tuberculosis* antigens. This is the first report of ICL as a candidate target for the serological diagnosis of TB.

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Conflicts of interest: none declared.

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RESUME

CONTEXTE : Mexico City, Méxique.
OBJECTIF : Identifier les protéines synthétisées par *Mycobacterium tuberculosis* en culture hypoxique qui ressemblent davantage à l'environnement d'un granulome qu'une culture aérobie et déterminer si elles sont reconnues par les anticorps des patients atteints de tuberculose pulmonaire (TBP) active.
SCHÉMA : Des extraits solubles de *M. tuberculosis* H37Rv en culture dans des conditions aérobies ou hypoxiques ont été analysés par électrophorèse sur gel de polyacrylamide en deux dimensions, et les protéines surexprimées sous hypoxie ont été identifiées par spectrométrie de masse. La présence d'anticorps immunoglobuline (Ig) G, IgA et IgM dirigés contre ces protéines a été déterminée dans le sérum de 42 patients atteints de TBP active et de 42 témoins en bonne santé.
RÉSULTATS : Nous avons sélectionné trois protéines de *M. tuberculosis* H37Rv (protéine alpha cristalline [Acr, Rv2031c], protéine universelle de stress Rv2623 et isocitrate lyase [ICL, RV0467]), qui ont été surexprimées sous hypoxie. Les titres d'anticorps IgA anti-Acr et anti-ICL ont été plus élevés chez les patients que chez les témoins sains, avec une aire sous la courbe de la fonction d'efficacité du récepteur de 0,71 pour les anticorps anti-IgA ICL.
CONCLUSION : ICL pourrait être utilisé en combinaison avec d'autres antigènes de *M. tuberculosis* afin d'améliorer la sensibilité et la spécificité des méthodes de diagnostic sérologique de la TB en cours.

RESUMEN

MARCO DE REFERENCIA: Ciudad de México, México.
OBJETIVO: Identificar las proteínas sintetizadas por *Mycobacterium tuberculosis* en cultivos bajo condiciones de hipoxia que simulan el ambiente del granuloma de mejor manera que los cultivos aeróbicos, y determinar si estas proteínas son reconocidas por anticuerpos de pacientes con tuberculosis pulmonar (TBP) activa.
MÉTODOS: Los extractos solubles obtenidos de *M. tuberculosis* H37Rv cultivado en condiciones aeróbicas o de hipoxia fueron analizados mediante electroforesis en geles de poliacrilamida bidimensionales, y las proteínas sobre-expresadas en condiciones de hipoxia se identificaron por espectrometría de masas. Posteriormente se estudió la presencia de anticuerpos de la clase inmunoglobulina (Ig) G, IgA e IgM contra estas proteínas en el suero de 42 pacientes con TBP activa y 42 controles sanos.
RESULTADOS: Se seleccionaron tres proteínas de *M. tuberculosis* H37Rv (proteína alfa-cristalina [Acr, Rv2031c], Rv2623 proteína de estrés universal y la isocitrato liasa [ICL, RV0467]) que estaban sobre-expresadas en condiciones de hipoxia. Los títulos de anticuerpos de la clase IgA anti-Acr y anti-ICL fueron mayores en los pacientes que en los controles sanos, con un área bajo la curva característica operativa del receptor de 0,71 para los anticuerpos IgA anti-ICL.
CONCLUSIÓN: La proteína ICL podría ser utilizada, en combinación con otros antígenos de *M. tuberculosis*, para mejorar la sensibilidad y especificidad de los métodos de diagnóstico de TB serológica actuales.