



Contents lists available at ScienceDirect

Tuberculosis

journal homepage: <http://intl.elsevierhealth.com/journals/tube>

CCL2, CCL18 and sIL-4R in renal, meningeal and pulmonary TB; a 2 year study of patients and contacts

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ARTICLE INFO

Article history:

Received 20 October 2010

Received in revised form

23 December 2010

Accepted 28 December 2010

Keywords:

Pulmonary tuberculosis

Meningeal tuberculosis

CCL2

CCL18

sIL-4R

Renal tuberculosis

SUMMARY

The role of Th2 cytokines and Th2-associated chemokines in tuberculosis (TB) remains controversial, though in Mexico a polymorphism causing increased production of CCL2 is a risk factor. We studied levels of the Th2-associated chemokines CCL2 and CCL18, circulating soluble IL-4 receptors (sIL-4R), IL-4 and the inhibitory splice variant of IL-4 (IL-4δ2) in a cohort of patients with pulmonary TB and their healthy contacts. These were followed for 2 years during which time 10 contacts developed pulmonary TB. Results were compared with measurements made in renal and meningeal TB, and in disease controls with bacterial pneumonias or Dengue fever that have large Th2 components. In these disease controls both chemokines were significantly raised. They were also very significantly raised in all forms of TB, irrespective of age or disease site. Levels of CCL18 were raised least in meningeal TB, and most in pulmonary patients with long histories, when levels were similar to those in disease controls. Levels of CCL2, although also raised in all three forms of TB, were negatively correlated with CCL18. We found that levels of sIL-4R were strikingly reduced in all forms of TB, particularly meningeal.

Contacts who progressed could not be distinguished from contacts who remained healthy at 2 years in terms of IL-4, sIL-4R, CCL2 or CCL18. However contacts had raised expression of IL-4δ2 as previously found. These results indicate vigorous and previously unrecorded activity within the Th2 axis, and further investigation is warranted.

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1. Introduction

The role of Th2 cytokines in human tuberculosis (TB) is controversial, but the issue is important because IL-4 and IL-13 are known to inhibit the effector mechanisms that control *Mycobacterium tuberculosis* (cytotoxic lymphocytes [CTL],¹ apoptosis, autophagy,² and macrophage activation³), and to drive pulmonary fibrosis.⁴ The latter is a crucial but neglected aspect of tuberculosis. In the mouse the role of IL-4 depends on the challenge dose used to infect the animals. IL-4 is not expressed at increased levels in mice infected with low doses of *M. tuberculosis* by aerosol, and the animals are not more resistant following knockout of the genes encoding IL-4 and IL-13.⁵ On the other hand, if mice are infected with larger doses, whether by instillation into the airways^{6,7} or by i.v. injection,⁸ IL-4

expression rises to high levels. Moreover IL-4 KO experiments,⁶ or neutralisation of the IL-4 *in vivo* with antibodies,⁹ attenuate the disease after high dose challenge, demonstrating that in this situation IL-4 plays a significant detrimental role.

Interestingly, high levels of IL-4 in tuberculosis patients are most often reported in developing countries,¹⁰ including those in South America.^{11,12} Much TB in developing countries occurs in individuals who are partially immunised by exposure to environmental mycobacteria and/or BCG, and it is likely that in many individuals the disease develops following prolonged high dose challenge from family members in crowded living conditions. Thus a subset of patients in developing countries might resemble the mouse models after high dose challenge.

Resolving this issue has been hampered by the fact that IL-4 is difficult to measure, and biologically active at very low concentrations. Moreover it is not clear whether the currently available ELISA assays measure only IL-4, or also the antagonistic splice variant, IL-4δ2. These have opposing biological effects, but can only be distinguished by RT-PCR.^{13,14} The distinction matters because

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increased expression of the latter (i.e. IL-4 δ 2) is associated with stable latent infection that does not progress¹⁵ and also with recovery from TB following treatment.^{13,14}

In order to cast light on this situation we have measured IL-4 δ 2 and other parameters involved in the regulation or function of Th2 responses in cohorts of Mexican patients with pulmonary, renal or meningeal TB. The data were compared with values obtained from healthy contacts, and from controls with other bacterial lung infections or with dengue fever (classical or haemorrhagic). The contacts were followed up for 2 years, and 10 new TB cases developed in the contact population. These individuals were analysed separately. We included chemokine (C-C motif) ligand 2 (CCL2, also known as MCP-1) because it is regarded as a “Th2 chemokine”,¹⁶ and a single nucleotide polymorphism of CCL2 that results in increased levels was reported to affect susceptibility to TB in Mexico.¹⁷ We also included CCL18 because its expression is induced in alveolar macrophages, DC, and monocytes by IL-4 and IL-13, whereas it is inhibited by IFN- γ .^{3,18,19} These findings define CCL18 as a marker of alternative macrophage activation.^{3,19} CCL18 is chemotactic for Th2 cells, and a potent regulator of pulmonary fibrosis.^{18,19} Finally, we measured soluble IL-4 receptors (sIL-4R). These are produced either by secretion of an alternatively spliced variant by cells stimulated by IL-4, or by proteolytic cleavage of the membrane-bound receptor after TCR engagement.²⁰ sIL-4R can inhibit IL-4 at high concentrations, but it can also enhance the actions of IL-4. It has a lower affinity for IL-4 than the membrane form of the receptor, so sIL-4R can protect circulating IL-4, prolong its $\frac{1}{2}$ life 10- to 20-fold, and pass it on to the membrane receptors.²¹

2. Material and methods

2.1. TB study populations and contacts

The study population consisted of pulmonary tuberculosis patients being treated at the Universidad Veracruzana's Health Clinic in Coyopolan, Veracruz, Mexico. All participants gave fully informed written permission before entry into the study. Sera and sputa were collected from February 2008 to November 2009. Samples were obtained from 34 patients with clinical pulmonary TB associated with abnormal respiratory function, afternoon fever and loss of weight. The disease was confirmed by lung radiology and by identification of tuberculosis bacilli in sputum smears using the Ziehl–Neelsen method. Further confirmation of the diagnosis of active TB was obtained using an in-house multiple PCR method (MPCR) that will be described in detail elsewhere. Briefly DNA was extracted both from the sputum and from the blood samples. The assay targets genes that are present in multiple copies in the genome of *M. tuberculosis* (IS6110, IS1081, and genes encoding the Ag 85 complex). The assay therefore yields up to three bands of 375 bp, 248 bp and 162 bp respectively. All patients designated as pulmonary TB recruited into this study were positive by this method. Eighteen of the 34 patients had been in therapy with a standard anti-tuberculosis regimen that included RMP, PZA and INH daily for between 4 months and 1 year. The other 16 patients were without treatment at the time of recruitment. Five of these, with durations of disease between 2 and 7 years, had received incomplete treatment or had defaulted.

Most of the pulmonary TB patients also had parasitic infections, and these were identified and recorded (*Ascaris* spp. (14 patients), *Enterobius vermicularis* (5), *Trichuris trichiura* (4), *Hymenolepis nana* (2), *Uncinaria* (1)). This was not recorded for the other groups.

Nineteen cases of tuberculous meningitis (12 males and 7 females) were also studied. Patients ranged in age from 4 to 89 years with an average age of 38. The patients had a 2–3 week history of clinical symptoms such as headache, malaise, mental confusion,

spontaneous vomiting and intracranial hypertension. Additional support for the diagnosis was derived from lumbar punctures: examination of CSF revealed pleocytosis made up of a mixture of polymorphonuclear and mononuclear cells, strikingly elevated proteins and moderately reduced glucose. Confirmation of the diagnosis was obtained by the MPCR method outlined above, using DNA extracted from CSF and from blood. All patients designated as meningeal TB recruited into this study were positive by this method.

Another group of 21 patients (9 males and 12 females) were diagnosed as renal TB. They ranged in age from 14 to 65 years with an average age of 49. They presented with symptoms of renal TB such as chronic and recurrent fever, back pain, in some cases pyuria, weight loss, malaise, chills, and loss of appetite. Radiological studies showed asymmetrically decreased kidney size and deformity in the calyceal system. Confirmation of the diagnosis was obtained by the MPCR method outlined above, using DNA extracted from urine and from blood. All patients designated as renal TB recruited into this study were positive by this method. The renal and meningeal TB patients were all new patients, with no previous treatment.

2.2. Contacts

In addition, sera were obtained from 39 household contacts of the pulmonary TB cases. (Contacts of the meningeal and renal TB cases were not recruited). The mean age of the contacts was 30 yrs. They were all without clinical evidence of the disease. When tested for *M. tuberculosis* by MPCR in blood, 22 were positive and 17 negative. In a two year follow-up at the clinic, 10 of the 39 developed active pulmonary tuberculosis. The diagnosis of TB in these contacts was based on the same criteria as the original 34 patients. Two progressors came from the MPCR+ group, 8 from the MPCR– group. Thus MPCR-positivity at time 0 did not help to predict subsequent progression to pulmonary disease.

2.3. Disease controls

2.3.1. Bacterial pneumonias

Sera were obtained from patients at the Civil Hospital, SSA in Xalapa, Veracruz. The patients were between 5 and 67 years old with a mean age of 40. These individuals were characterised by high fever, headache, cough, muscle aches, pain in the legs, and mismatching ventilation with a clinical history of 5–7 days. Roentgenographically the area of lung involvement was diffuse. In 32 patients the involvement was unilateral while in 8 patients it was bilateral. The sputum contained numerous neutrophils and pyogenic bacteria.

2.3.2. Dengue

The clinical symptoms of the 12 patients studied with classical dengue were fever >39.3 °C for 3–5 days, headache, muscle and joint pain, weakness and a rash followed by remission of the fever. Sera were obtained from the 12 patients for further study. The detection and typing of dengue virus in the acute phase was done by nested RT-PCR. The results were 7 T2, 3 T3 and 2 T4 type. None had T1. Haemorrhagic dengue in 17 patients was characterised by a similar initial course. They presented hemorrhagic manifestations such as cutaneous bleeding in the form of pinpoint hemorrhages (petechiae). These lesions became confluent and gave rise to ecchymoses nosebleeds, bleeding of the gums, melena, hematuria and circulatory insufficiency. Laboratory findings were thrombocytopenia, leukopenia and haemoconcentration. The molecular diagnosis in the 17 patients was done by nested RT-PCR that showed 2 subtypes of dengue virus, representing a secondary infection with another subtype. Fifteen patients had T3-T2, 1 patient had T2-T4 and one patient had the T1-T2 subtypes.

2.3.3. Enzyme-linked immunosorbent assays (ELISA)

Serum samples were screened by enzyme-linked immunosorbent assay (ELISA) using commercial kits provided by R&D Systems for measuring IL-4, IL4-sR, CCL18 and CCL2. (Since no ELISA kit is available for IL-4 δ 2 this cytokine was measured by reverse transcriptase polymerase chain reaction (RT-PCR) only (described below).

2.3.3.1. RT-PCR. Total mRNA was isolated from 1 ml of blood dissolved with 1 ml Trizol plus 100 μ l chloroform following the manufacturer's recommendations. Quality and quantity of RNA were evaluated by spectrophotometry (A260/280) and on agarose gels. Five micrograms of mRNA were reverse transcribed using the Omniscript kit (Qiagen, Inc) in combination with oligo dT. Real-time PCR was performed using the 7500 real-time PCR system (Applied Biosystems, USA) and Quantitect SYBR Green Mastermix kit (Qiagen). Standard curves of quantified and diluted PCR product, as well as negative controls, were included in each PCR run. Specific primers were designed using the program Primer Express (Applied Biosystems, USA) for IL4 and IL4- δ 2: forward 5'-CAGGAGAAGGGAACACCAC-3', IL-4 reverse 5'-GCTGTTTAGCTTCCAGGAAG-3', and IL-4 δ 2 reverse 5'-TGCATGTGCCAAACGTCC-3'. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH): forward 5'-CATTGTGGAAGGGTCATGA-3', reverse 5'-GGAAGGCCATGCCAGTGAGC-3'. Cycling conditions used were: initial denaturation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min. Quantities of the specific mRNA in the sample were measured according to the corresponding gene specific standard. The mRNA copy number of each cytokine was related to one million copies of mRNA encoding the G3PDH gene.

2.3.4. Statistical analysis

All analysis was performed in GaphPad PRISM, using the Kruskal–Wallis, Dunn's multiple comparison tests, and Spearman's two-tailed non-parametric correlation as described in the legend of each figure.

3. Results

3.1. Cytokine and chemokine levels by ELISA

The serum levels of IL-12 did not differ between the groups (data not shown).

All three types of TB patient also had raised levels of CCL2 and CCL18 compared to both contact groups (Figure 1A and B).

Interestingly renal TB was not significantly different from pulmonary TB in terms of these chemokine levels, but meningeal TB had lower CCL18 ($p < 0.05$) and higher CCL2 ($p < 0.001$). Similarly the ratio of CCL18/CCL2 was lower in meningeal (10.1+/-4.6) than in pulmonary TB (55.7+/-54.9; $p < 0.0001$, Mann–Whitney; data not shown). The levels of CCL18 seen in pulmonary and renal TB were comparable to those seen in haemorrhagic (1348+/-341) and classical (1154+/-69.83) dengue (data not shown) or in non-tuberculous pneumonias (Figure 1B).

By these parameters the 10 previously healthy contacts that subsequently developed active pulmonary TB did not differ significantly from those who remained disease-free.

The low ratio of CCL18 to CCL2 in meningeal TB could reflect the fact that CCL18 is highly expressed in human lung.¹⁹ On the other hand the meningeal TB patients had considerably shorter durations of disease (2–3 weeks) than the pulmonary patients, some of whom were long-term treatment failures and defaulters. Interestingly, pulmonary TB patients' CCL18 levels rose significantly with time ($p = 0.032$), while levels of CCL2 tended to fall. Thus there was a highly significant inverse correlation between the two chemokines ($p < 0.0001$; Spearman's non-parametric correlation) (Figure 2).

Analysis of the other clinical parameters available (age (the age range was very wide), weight loss, anorexia, haemoptysis, duration of treatment (if any), nocturnal fever, presence or absence of helminths in faecal samples) did not reveal other correlations after appropriate statistical corrections.

3.2. IL-4, sIL-4R and IL-4 δ 2

Levels of soluble IL-4 receptors were very significantly reduced in all three types of TB ($p < 0.001$), compared to both the initially healthy contact group that remained healthy, and to those that progressed (Figure 3A). Interestingly sIL-4R was significantly more reduced in meningeal than in pulmonary TB. The manufacturer stopped supplying the kits, so it was not possible to run this assay on samples from the patients with dengue or other bacterial infections.

IL-4 was also detectable in the sera from all the groups, but levels were similar in the three types of TB, and the contacts. The

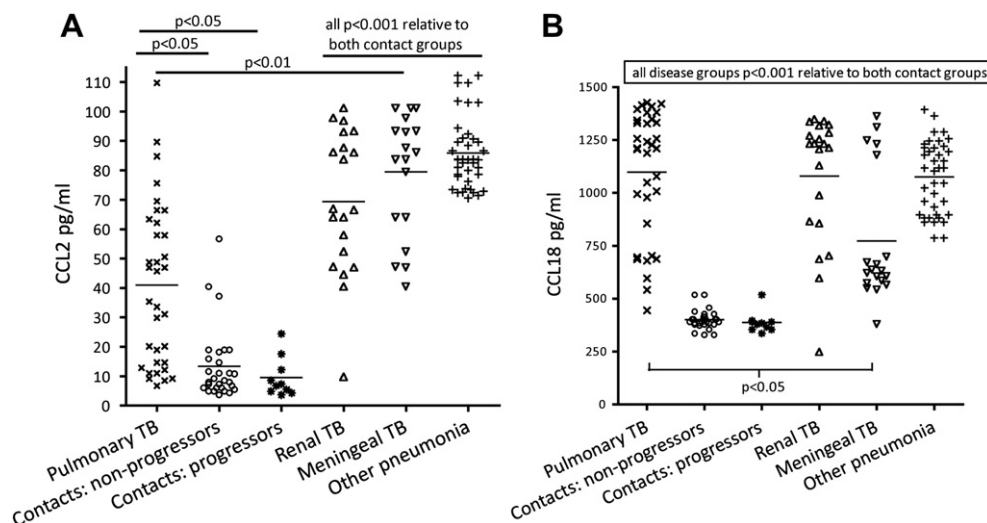


Figure 1. Serum levels of CCL2 and CCL18 in the serum of TB patients, contacts, and patients with other bacterial pneumonias. All three types of TB patient also had raised levels of CCL2 (A) and CCL18 (B) compared to both contact groups. Although raised relative to the contact groups, CCL18 in meningeal TB was significantly lower than in progressive pulmonary TB ($p < 0.05$), while CCL2 in this group was higher than in pulmonary TB ($p < 0.001$). All statistics by Kruskal–Wallis (means differ, $p < 0.001$, both graphs) and Dunn's multiple comparison tests.

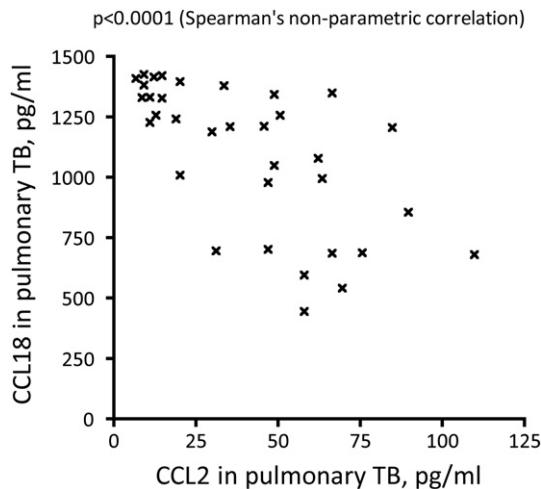


Figure 2. Levels of CCL2 and CCL18 in the serum of pulmonary TB patients. CCL2 and CCL18 are strongly inversely correlated ($p < 0.0001$). (Non-parametric Spearman's correlation, two-tailed).

patients with other bacterial infections (or with dengue; data not shown) showed significantly higher levels of IL-4 than did the TB patients (Figure 3B). Since there is no ELISA kit for the antagonist splice variant, IL-4 δ 2, this was assayed by RT-PCR. We had previously shown in a cohort of Ethiopians that raised IL-4 δ 2 is found in established stable latent infection.¹⁵ Figure 4 shows that the same is true in Mexico. mRNA encoding IL-4 δ 2 is increased in the peripheral blood mononuclear cells of PCR-positive contacts who did not develop disease, relative to PCR-negative contacts, and to TB patients. Interestingly however, it was also increased, (in 3 cases markedly so) in the group of contacts who subsequently developed disease. It should be noted that they were sputum-negative, and free of symptoms at the time the samples were taken. IL-4 δ 2 levels in TB patients were similar to those in PCR-negative contacts, indicating that as disease develops, expression of IL-4 δ 2 falls again. The 10 progressors did not differ from the other contacts by any other parameter.

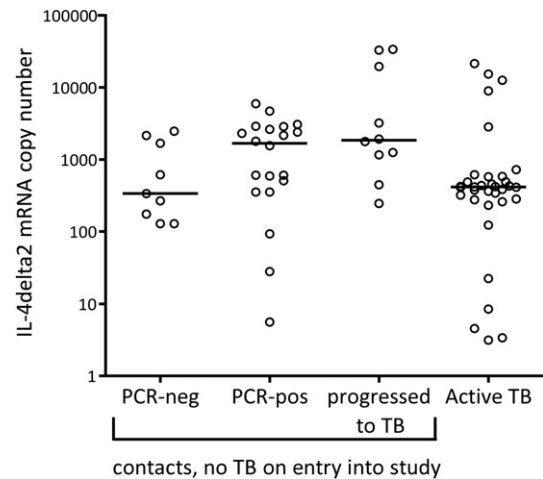


Figure 4. RT-PCR for mRNA encoding IL-4 δ 2. Levels in PCR-negative contacts, who did not progress to disease do not differ from levels in pulmonary TB patients. However levels are raised relative to these groups in the PCR-positive contacts who did not progress to TB, and in the 10 contacts who did progress to TB. Data are expressed as mRNA copy relative to one million copies of mRNA encoding G3PDH. Statistics by Kruskal–Wallis test (means differ, $p = 0.0085$), followed by Dunn's multiple comparison test. The 10 progressors did not differ from the other contacts by any other parameter.

4. Discussion

We have compared a number of parameters in TB patients and their contacts, all of whom were followed up for 2 years. We find striking increases in two chemokines, CCL2 and CCL18 that are both regarded as markers/promoters of Th2 activity. Genetic studies have implicated both CCL18²² and CCL2^{17,23–25} in susceptibility to TB, but they have received little attention. We also show for the first time that TB is accompanied by a fall in levels of circulating sIL-4R. Interestingly, these changes in CCL2, CCL18 and sIL-4R were found in all types of TB studied, and were unaffected by age, previous treatment, or parasite load. There was virtually no overlap with control groups.

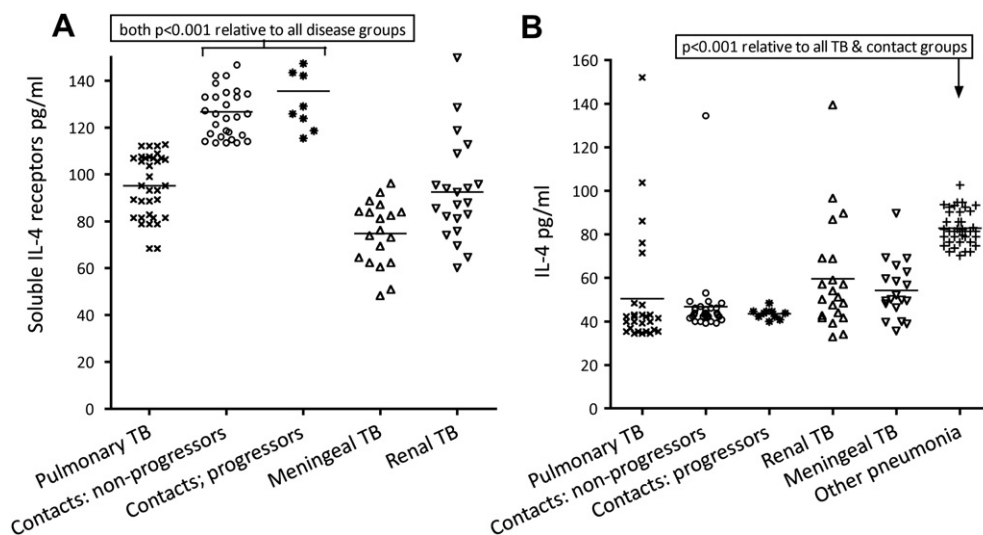


Figure 3. Levels of sIL-4R and IL-4 in the serum of TB patients, contacts, and patients with other bacterial pneumonias. sIL-4R are reduced in all TB groups relative to both groups of contacts ($p < 0.001$ in each case). The levels were lower in meningeal TB than in pulmonary TB ($p < 0.05$). IL-4 readily detectable in all contacts and TB patients, but levels did not differ between groups. However there was more IL-4 in the serum from other bacterial pneumonias than in any of the TB or TB-contact groups ($p < 0.001$ in every case). All statistics by Kruskal–Wallis (means differ, $p < 0.001$, both graphs) and Dunn's multiple comparison tests.

The increases in CCL18 were particularly striking. The increase was least in meningeal TB which involved patients with very short clinical histories, and highest in those pulmonary patients with very long histories, suggesting the possibility that CCL18 levels rise, and CCL2 levels fall over time. This could also account for the remarkable negative correlation between these chemokines shown in Figure 2. However confirmation of this hypothesis will require further studies.

We are not aware of any published studies of CCL18 in tuberculosis, though there is one report that *M. tuberculosis* can induce expression of CCL18 in human macrophages (including alveolar) and DC *in vitro*.²⁶ CCL18 is important in the current context because it is a marker of alternative macrophage activation.^{3,18,19} IL-4 and IL-13 induce expression of CCL18 in alveolar macrophages, DC and monocytes, whereas expression is inhibited by IFN- γ .^{3,18,19} The CCL18 receptor is preferentially expressed on basophils and Th2 cells, for which CCL18 is a chemoattractant.¹⁸ Thus CCL18 is associated with allergic asthma. PBMC from asthmatics allergic to house dust mite cultured in the presence of Der p1 allergen secreted CCL18 after stimulation, whereas those from healthy donors did not.¹⁸ Similarly CCL18 expression is associated with an atopic dermatitis phenotype when compared with other chronic inflammatory skin diseases.²⁷ Another major function of CCL18 is the regulation of fibrosis. In patients with fibrosing lung disease spontaneous CCL18 production by alveolar macrophages is greatly increased and correlates with CCL18 serum concentrations. Changes in these concentrations closely correlate with changes in pulmonary function data at follow-up.^{19,28} CCL18 shares several functional properties with TGF- β and upregulates collagen production by lung fibroblasts via the same signalling pathways.¹⁹ The levels of CCL18 seen in long-term TB were similar to those seen in conditions with large Th2 components, such as bacterial pneumonias and dengue fever. These results suggest that as TB progresses there is an increasing Th2 component that might contribute to the pulmonary fibrosis and to deactivation of Th1-mediated effector pathways. It is however possible that *M. tuberculosis* can trigger release of CCL18 via pathways that do not involve Th2 cytokines.

A similar dilemma applies to our finding that CCL2 is also increased in all types of TB in Mexico. There are reports that *M. tuberculosis*, BCG or specific recombinant antigens can induce secretion of CCL2 from the peripheral blood mononuclear cells of TB patients.^{29,30} One might therefore expect to see raised serum levels in TB, and this was found in this study and in a previous study in Mexico,¹⁷ but not in Pakistan³¹ or Brazil.³² Our data cast some light on these discordant results because CCL2 was most obvious in early disease (and in meningeal disease) and was inversely related to levels of CCL18, which rise with increasing disease duration. Thus discordant data might be related to disease duration in different studies.

CCL2 is produced by many cell types, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic and microglial cells.³³ CCL2 regulates the migration and infiltration of monocytes, memory T lymphocytes and natural killer (NK) cells. It also has poorly studied anti-inflammatory roles dependent on expression of its receptor (CCR2) in regulatory T cells.^{33,34} CCL2 is often designated a Th2 chemokine because, in the mouse at least, CCL2 is both a cause and a consequence of Th2 bias. IL-4 induces the production of CCL2,³ polarises Th0 cells towards Th2^{35–37} and preferentially recruits Th2 cells.³⁸ Moreover CCL2 appears to downregulate release of IFN- γ from Th1 cells.³⁹

The same is usually true in man. IL-4 and IL-13 upregulate expression of CCL2 in human bronchial epithelial cells,³⁸ and CCL2 is strikingly upregulated in biopsies from asthmatics.⁴⁰ In sharp contrast, CCL2 downregulates release of IL-12 from human

macrophages.⁴¹ Importantly, serum levels have been measured in a number of human diseases, and are characteristically raised more in Th2 or mixed Th1/Th2 disorders than in Th1-mediated disorders.¹⁶

Recent studies of chemokine CCL2 in TB patients in Mexico lend some support to this view.¹⁷ CCL2 is raised in Mexican TB patients, and susceptibility to the disease is linked to a single nucleotide polymorphism (SNP) that leads to increased production. Patients with this SNP had higher CCL2 levels, but decreased levels of IL-12 relative to patients with the other variants.¹⁷ The effect of this SNP on susceptibility appeared to be very strong,⁴² and similar results have been reported in some^{23,24} but not all studies,^{22,43} though in one case a link to a different SNP was found.²⁵ Interestingly the TB-linked polymorphism described in Mexico has been associated with asthma in Europe.⁴⁴

An obvious hypothesis is that early release of CCL2 recruits Th2 cells, which then release Th2 cytokines such as IL-13 and IL-4, driving local production of CCL18.

Although we did not find any difference between IL-4 levels between TB patients and healthy contacts, we did note that circulating levels of soluble IL-4 receptors (sIL-4R) were markedly reduced in all types of TB, particularly in meningeal disease. sIL-4R is produced either by secretion of an alternatively spliced variant by cells stimulated by IL-4, or by proteolytic cleavage of the membrane-bound receptor after TCR engagement.²⁰ sIL-4R can inhibit IL-4 at high concentrations, but it can also enhance the actions of IL-4. It has a lower affinity for IL-4 than the membrane form of the receptor, so sIL-4R can protect circulating IL-4, prolong its $\frac{1}{2}$ life 10- to 20-fold, and pass it on to the membrane receptors.²¹ Therefore the biological significance of the falling sIL-4R levels in TB is not clear. However when set against the few existing studies in man, the result is provocative. Levels of sIL-4R were elevated in children with severe malaria⁴⁵ and in visceral leishmaniasis,⁴⁶ but as we have found in our TB patients, levels were abnormally low in subjects with stable asthma⁴⁷ or allergic rhinitis.⁴⁸ Unfortunately our study is limited by the fact that the manufacturer stopped producing the kits, and we were unable to test the sera from the bacterial pneumonias or from dengue in which there were striking increases in IL-4.

Finally we have confirmed again¹⁵ that mRNA encoding IL-4 δ 2, the inhibitory splice variant of IL-4, is increased in peripheral blood mononuclear cells of subjects with stable latent TB when compared to PCR-negative contacts who remained healthy, but is low in patients with progressive pulmonary TB. However a subset of 10 contacts who progressed to active disease within 2 years of recruitment also had raised IL-4 δ 2, suggesting that it is also a marker of very early subclinical infection, and cannot be regarded as indicating that progression will not occur.

The rate of progression of contacts to pulmonary TB (10 out of 39) was very high, but there are no other data from this part of Mexico for comparison. Contacts are not given prophylaxis in Mexico, and the contacts lived in the same small crowded homes as the index cases. The fact that no individuals designated healthy contact at time of entry into the study had raised CCL2 or CCL18, or reduced sIL-4R, implies that they were correctly classified.

In conclusion, rather than studying conventional Th1-linked mediators, we have studied mediators associated with Th2 activity. The data have revealed previously unrecorded changes in CCL18 and sIL-4R, and an inverse correlation between CCL18 and CCL2, suggesting that the latter might be released early, followed later by CCL18. These results suggest that TB is accompanied by significant changes in the regulation of Th2-mediated events. The study is purely correlational and cannot cast light on the relevance of the role of the mediators in the disease, but since these findings are backed by previously published genetic findings,^{17,22} our results highlight the need for further studies.

Funding: The project was funded by the Fundacion Miguel Aleman Valdez.

Competing interests: The authors have no competing interests.

Ethical approval: The study was approved by the appropriate ethical committee. All subjects gave fully informed written consent.

Acknowledgements

We gratefully acknowledge the help of the patients and their families, and of numerous ancillary hospital and laboratory staff.

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