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REVIEW

Cytokines for monitoring anti-tuberculous therapy: A systematic review

Vanessa Clifford [a], Christel Zufferey [a], Alan Street [b], Justin Denholm [b], Marc Tebruegge [a, c], Nigel Curtis [a, *]

[a] Department of Paediatrics, The University of Melbourne and Murdoch Children's Research Institute, Royal Children's Hospital Melbourne, Parkville, VIC, Australia
[b] Victorian Infectious Diseases Unit, The Royal Melbourne Hospital, Parkville, VIC, Australia
[c] Academic Unit of Clinical and Experimental Medicine, Faculty of Medicine & Respiratory Biomedical Research Unit & Institute for Life Sciences, University of Southampton, United Kingdom

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SUMMARY

The ability to monitor response to therapy for tuberculosis (TB) and confirm adequate treatment would be a major advance. The low reversion rate of interferon-gamma based assays means that they are unlikely to be useful for monitoring therapy. Several exploratory studies have evaluated the diagnostic potential of cytokine biomarkers other than interferon-gamma for monitoring anti-tuberculous therapy. A systematic review of these studies was performed to identify the most promising candidate biomarkers. TNF-α, IL-2, IL-6, IL-10 and IL-12 were the most extensively investigated cytokines. There was significant heterogeneity between studies in relation to study design and laboratory methodology, complicating direct comparisons. There was marked variation between studies in the observed changes during treatment for many of the biomarkers. Further longitudinal studies in sufficiently large patient cohorts with rigorous methodology are needed to determine the true potential of individual cytokine biomarkers, or combinations, for monitoring TB treatment.

1. Introduction

Mycobacterium tuberculosis (MTB) infection remains a major challenge globally. It is one of the leading infectious causes of death worldwide [1], responsible for an estimated 1.7 million deaths each year [2]. Adequate therapy requires prolonged treatment resulting in significant cost and use of medical resources. In addition, studies suggest that patients' adherence to treatment declines with extended courses [3].

The ability to monitor the response to treatment for tuberculosis (TB) and confirm adequate treatment would be a major advance with a number of significant benefits. In particular, it would allow comparisons of different antibiotic regimens and evaluation of shorter treatment durations. Currently, TB drug treatment trials are difficult and expensive because the absence of reliable surrogate markers of treatment success means trials require prolonged follow up to detect the small proportion of participants who subsequently develop TB. The ability to confirm adequate treatment would also help reduce transmission from subsequent reactivation following inadequate treatment. Moreover, a robust biomarker would be particularly useful to confirm adequate therapy of latent TB, for which there are currently no diagnostic tools. Finding biomarkers of successful treatment might also provide insights into surrogate markers of protective immunity against TB.

There has been recent interest in the possibility of using interferon-gamma (IFN-γ) release assays (IGRAs) for monitoring TB treatment. Existing studies, however, have yielded conflicting results [4–9]. A recent systematic review by Chiappini et al. highlighted the considerable inconsistency in the reported effect of anti-tuberculous treatment on the kinetics of IGRA results, and estimated that the overall reversion rate (ie change in categorical results from 'positive' to 'negative') of the QuantiFERON Gold in-Tube (QFT-G-IT) assay at the end of TB treatment was only 30% [10]. This low reversion rate suggests that IFN-γ-based assays are unlikely to be useful for monitoring the response to treatment.

* Corresponding author. Department of Paediatrics, The University of Melbourne, Royal Children's Hospital Melbourne, Flemington Road, Parkville, VIC 3052, Australia. Tel.: +61 3 9345 6366; fax: +61 3 9345 4751.
E-mail address: nigel.curtis@rch.org.au (N. Curtis).

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In recent years, several exploratory studies have evaluated the diagnostic potential of cytokine biomarkers other than IFN-γ for monitoring anti-tuberculous treatment. We did a systematic review of these studies to identify the most promising candidate biomarkers, and provide a critical analysis of the supporting data for each.

2. Methods

2.1. Search strategy

This review was conducted in accordance with the 'preferred reporting items for systematic reviews and meta-analyses' (PRISMA) statement. Original articles, letters to the editor and published abstracts were identified by searching MEDLINE (1947 to June 2014), EMBASE (to June 2014), and the Cochrane Central Register of Controlled Trials (CENTRALL). The following search terms were used: (exp *Mycobacterium tuberculosis*/an, ch, im, ph or exp *Tuberculosis/bl, di, im, mi, ph, pp) and (exp *"intercellular signaling peptides and proteins"/or exp *cytokines/or exp chemokines/or exp interferons/or exp interleukin 1 receptor antagonist protein/or exp interleukins/or exp lymphokines/or exp monokines/or exp tumor necrosis factors/). Reference lists of identified relevant publications were also hand-searched for further relevant publications.

2.2. Study selection

Titles and abstracts of all publications were reviewed to select studies for full text review. To meet inclusion criteria, studies had to report the use of a cytokine biomarker other than IFN-γ in blood for monitoring TB treatment in humans [10] and include only participants receiving appropriate anti-tuberculous treatment. No restrictions were placed on sample size or method of data collection. Case reports and meeting abstracts, as well as studies without original data. To provide a focussed review, we excluded studies of other potentially important aspects of the host response to *M. tuberculosis*, such as gene expression, microRNAs, stable non-coding RNAs and host metabolomics (e.g. mass spectrometry for small, water soluble metabolites and/or lipid metabolites) and proteonomics.

2.3. Data extraction

For all eligible studies, the following data were extracted using a standardised collection sheet: country of study, age group of participants (categorised as children, adolescents or adults), TB status of participants (confirmed/probable active TB, or latent TB infection), immune status (including HIV infection), study design (longitudinal or cross-sectional), cytokines measured, stimulation used, incubation time(s), measurement method (ELISA, flow cytometry) and change in measured cytokine concentrations during treatment. Active TB was categorised as either 'confirmed' (culture or PCR positive) or 'probable' (clinical or histological diagnosis of TB with appropriate response to treatment).

3. Results

Of the 5033 articles screened by title and abstract, 4985 were excluded. This left 49 potentially relevant articles. A further 17 articles that did not meet the inclusion criteria were excluded [11–27], leaving 32 publications [28–59] (Figure 1).

Seventeen studies investigated cytokine responses after MTB-specific *in vitro* stimulation of blood (Table 1) [28–44], and 15 studies measured *ex vivo* serum cytokine concentrations without a stimulation step (Table 2) [45–59]. The overall results are summarised in Table 3.

3.1. Stimulated cytokine responses

Of the 17 studies that investigated cytokines after *in vitro* stimulation, eight were small pilot studies with fewer than 10 [28,31,42,43] or fewer than 20 [29,32,34,38] patients with active TB. Six of the studies were cross-sectional [29,35,37,38,40,44], rather than longitudinal.

The largest studies that followed patients longitudinally over the course of treatment were by Eum et al. [30] (38 participants with active TB, with blood samples collected after 0, 2, 6 months of treatment) and Sai et al. [39] (123 participants with active TB, with blood samples collected after 0, 2, 4, 6 months of treatment). Other relatively large longitudinal studies were those by Millington et al. (23 participants with active TB) [36] and Singh et al. [41] (21 participants with active TB).

All studies addressed changes in cytokine responses during treatment in patients with active TB. There were no studies investigating cytokine changes during treatment for latent TB infection. All studies included a control group (usually healthy controls) to compare cytokine responses at baseline (ie start of treatment) [28–44].

The cytokine biomarkers investigated in the included studies comprised TNF-α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17 and IP-10. Several studies investigated more than one cytokine biomarker for monitoring TB treatment (Table 1). The most studied biomarkers were TNF-α (8 studies), IL-2 (6 studies) and IL-10 (7 studies).

3.1.1. TNF-α responses

Eight studies evaluated the role of TNF-α responses induced by stimulation with mycobacterial antigens. The largest study, by Eum et al., using ELISA, reported an increase in TNF-α responses with treatment [30]. Five studies using flow cytometry [31,33,35,38,44], three of which were cross-sectional [31,38,44], reported a reduction in TNF-α responses. Of note, two of these studies also reported an increase in polyfunctional T cells (producing TNF-α and other cytokines simultaneously) at the end of treatment compared to that at treatment initiation [31,44].

Two studies, one using ELISA and the other flow cytometry, found no change in TNF-α responses during treatment [28,37].

3.1.2. IL-2 responses

Six studies assessed the role of IL-2 responses in monitoring treatment. Of these, three were cross-sectional studies [38,40,44]. Five studies used flow cytometry to assess cytokine responses [31,36,38,40,44]. Two studies reported lower IL-2 responses at the end of treatment, compared to start of treatment [38,60] and one study reported increasing frequency of IL-2 producing TB-specific T cells during treatment [36]. All five studies found an increase in polyfunctional T cells producing IL-2 and other cytokines at the end of treatment [31,36,38,40,44]. One small pilot study (including only 6 participants), using xMAP *Luminex* to determine cytokine levels, found no change in IL-2 responses with treatment [28].

3.1.3. IL-10 responses

Seven studies assessed the role of IL-10 responses in monitoring treatment. Of these, two were cross-sectional studies [35,37]. Three studies found a reduction in IL-10 responses with treatment [39,41,43], one study found an increase [30] and three found no change [28,35,37]. Amongst the larger, better-designed studies, Eum et al. found an increase in IL-10 with treatment and Sai Priya et al. found a reduction [39]. These studies used different antigens for stimulation: Sai Priya et al. used the 32kd antigen of