

IL-17 and IFN- γ production in peripheral blood following BCG vaccination and *Mycobacterium tuberculosis* infection in human

Q. LI^{1,2,3}, J. LI³, J. TIAN⁴, B. ZHU², Y. ZHANG⁵, K. YANG⁴, Y. LING³, Y. HU¹

¹College of Veterinary Medicine, Gansu Agriculture University, China

²Lanzhou Center for Tuberculosis Research, School of Basic Medical Science, Lanzhou University, China

³Institute of Pathophysiology, School of Basic Medical Science, Lanzhou University, China

⁴Department of Evidence-Based Medicine, School of Basic Medical Science, Lanzhou University, China

⁵Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, USA

Abstract. – **BACKGROUND:** During *Mycobacterium tuberculosis* (Mtb) infection, cells of the immune system rely on cytokines to regulate the activity of other immune and structural cells such as IFN-gamma and IL-4. Recent studies suggest that Th17 cells secreting IL-17 may play a potential role in tuberculosis (TB) development.

AIM: To assess the effect of IL-17 on TB development, we provide a systematic review on the production of IL-17, IFN-gamma and IL-4 in infants or children vaccinated with BCG and in TB patients.

MATERIALS AND METHODS: The literature relevant with IL-17 and IFN-gamma production with or without IL-4 on human TB was retrieved from PubMed, EMBASE, Cochrane Library, BIOSIS Previews and the China Biomedicine Literature Databases (CBM) using the search terms “Interleukin-17 or Th17 cells” and “Tuberculosis”. The information of included studies, the production of IL-17 and IFN-gamma responding to antigens in the peripheral blood *in vitro*, was independently extracted by the first two researchers and subsequently qualitatively analyzed.

RESULTS: Nine studies from a total of 226 retrieved publications met the criteria. These included studies showed that BCG vaccination induced dramatically high level of IL-17 similar to IFN-gamma; The level of IL-17 and IFN-gamma were low while IL-4 was high in patients with active TB; IL-17 and IFN-gamma had a similar trend of increase during the conversion from active to latent TB while IL-4 inclined to decrease in this process.

CONCLUSIONS: IL-17 acts as an effector molecule similar to IFN-gamma after BCG vaccination and Mtb infection to protect human against TB. The current findings do not support IL-17 as an inducer of tissue damage in TB.

Key Words:

Tuberculosis, Interleukin-17, Th17 cells, IFN- γ , Th1 cells, Interleukin-4.

Introduction

TB is a leading infectious disease in the world partially due to little understanding of immunologic mechanism. So it is necessary to understand what constitutes protective immunity during natural infection with Mtb.

Mtb is phagocytosed by mononuclear macrophages and dendritic cells after invasion. And then the bacteria settle down in the phagosomal compartment by prevention from phagosome acidification or evasion of phagosomal effector mechanisms which can lead to a latent or active infection. During Mtb infection, MHC class II and I restricted CD4 and CD8, CD1-restricted and $\gamma\delta$ T lymphocytes are activated by protein antigens through MHC class II and I molecules, lipid antigens through CD1 molecules and phospholipids through $\gamma\delta$ T cells respectively. Activated T lymphocytes release IFN- γ and other cytokines which will activate macrophage to eliminate bacteria¹. CD4 T lymphocytes are differentiated into several different effector cells such as Th1, Th2, Th17 and regulatory T cells (Treg). Th1 cells mainly produce IFN- γ controlling intracellular infection including Mtb, whereas Th2 cells produce IL-4, IL-5 and IL-13 mediating humoral immunity. Treg produce IL-10 negatively regulating both IFN- γ and IL-17 responses². CD39-positive Treg inhibit generation and differentiation of Th17 cells via a latency-associated peptide-dependent mechanism³.

Th17 cells produce cytokines such as IL-17A (simply referred to as IL-17), IL-17F, IL-22, and IL-26 (in human). In addition, CD8 T cells, $\gamma\delta$ T cells and invariant natural killer T cells also pro-

duce IL-17. Th17 cells are positively regulated by TGF- β , IL-1 β , IL-6, IL-21 and IL-23 and negatively regulated by Th1 and Th2 cytokines⁴.

The ability of IL-17 to restrain Mtb is highly controversial. Mice deficient in IL-17 was unable to control Mtb with high dose intratracheal infection⁵ and adoptive transfer of Th17 cells partially inhibited Mtb growth⁶. Low Colony-Forming Units was detected in the lungs of C57BL/6 mice along with significantly higher numbers of Th17 cells⁷. Modified vaccinia virus Ankara expressing antigen 85A, a novel TB vaccine designed to improve the protective efficacy of BCG and in process of the second clinical study stage, induced co-production of IL-17 and IFN- γ by CD4 T cells from subjects⁸. On the other hand, IL-17 receptor knockout (KO) mice were not more susceptible than wild-type mice to Mtb pulmonary infection. Compared with Th1 cells, IL-17 showed limited roles in controlling host defense against Mtb in mice with a low dose aerosol infection⁹.

IL-17, specially, was estimated to mediate immune pathology in animal models of autoimmune diseases and infections, suggesting that IL-17 may make detrimental effect in TB pathology². Indeed, the increase of Th17 cells in the absence of IL-27 led to earlier death of animals despite increasing protection from Mtb¹⁰. Multidrug resistant TB patients showed the highest IL-17 expression with severe tissue damage, hinting IL-17-producing T cells could play an immunopathological role¹¹. However, multivariate analysis demonstrated that lower serum IL-17 level was associated with the mortality in patients with anti-TB treatment beyond two months¹². IL-17 in focus of infection was neither detected in patients with TB pericarditis or Mtb-infected and uninfected persons, which did not support a major role for IL-17 at established TB disease sites¹³.

IL-17 was recognized as an inducer of inflammation by induction of chemokines and accumulation of both polymorphic and mononuclear cells¹⁴ which could kill Mtb *in vivo* under appropriate environment or situation¹⁵. Inflammation can detect the changes in the metabolic status of the host caused by the infectious agents or the damage to the host tissues as sets of sensors¹⁶. IL-17 sounds an alarm and subsequently protects against infection or contributes to pathology by initiation of inflammation, accumulation of neutrophils and other mechanisms. In addition, Nandi and Behar¹⁷ indicated that IFN- γ inhibited the production of IL-17 by CD4 T cell, while Pasquinelli et al¹⁸ demonstrated that IL-17 inhibited IFN- γ production.

To evaluate the potential role of IL-17 and the relationship between IL-17/Th1 cells and IFN- γ /Th1 cells in human TB development, we screened all studies on human TB in which IL-17 and IFN- γ were detected and made a systematic review.

Materials and Methods

All papers on IL-17 and IFN- γ production with or without IL-4 in peripheral blood relevant with human TB were identified from PubMed, EMBASE, Cochrane Library, BIOSIS Previews and CBM up to 25 December 2011 using the search terms "Interleukin-17 or Th17 cells" and "Tuberculosis". If necessary, the Authors were contacted with the e-mail. Full-text articles were obtained from the internet and inter-library loan.

We precisely developed inclusive criteria because the bias mainly comes from selection of participants and performance of experiments of included studies. All participants were negative in HIV test. In the study on BCG-induced immunity, we included study that: (1) infants vaccinated with BCG at birth or 5-10 weeks of age; children, who had no previous BCG vaccination based on evidence of BCG scar or subsequent positive tuberculin skin test (TST), vaccinated at 12-13 years old, (2) immune response detected with ELISA at least 3 months after BCG vaccination, (3) whole blood inoculated with purified protein derivative (PPD) for 5 or 6 days. In the study on Mtb infection, we included study that: (1) all participants older than 15 years old, (2) active TB patients with clinical symptoms and acid fast smear or culture positive for Mtb, anti-TB treatment in 1-2 weeks or without treatment, no serious concomitant chronic conditions; for extra-pulmonary TB, acid fast bacillus positive for Mtb on biopsies or on pleural fluid, (3) latent TB subjects defined by a positive response in TST (two tuberculin units) with an induration of at least 10 mm diameter in majority of studies, or by the QuantiFERON TB Gold in tube (QFT)¹⁹ and Mtb-specific IFN- γ ELISPOT assay²⁰, (3) healthy donors (negative TST) with TST reaction size < 10 mm (< 5 mm in two studies^{21,22}), (4) healthy donors and Latent TB with normal chest radiographs, no pulmonary symptoms (cough, fever, chest pain, hemoptysis) or a positive sputum for Mtb by smear microscopy and culture, (5) PBMC or whole blood inoculated with antigen of Mtb or a mixture of phorbol myristate acetate (mitogen and T cell activator) and iono-

mycin (T cell activator), which can activate T helper cells²³, (6) the concentration of induced cytokines detected by ELISA or the percentage of activated T cell subsets analyzed by flow cytometry or ELISPOT (one study¹⁹).

The first two researchers screened the full text of potentially relevant studies for eligibility, extracted and checked the information of included studies independently. In case of doubt, more researchers reviewed the papers and reached a consensus decision. The systematic review was qualitatively analyzed due to the failure of asking for numerical data (mean and standard deviation or error) in most papers.

Results

Nine Studies Met the Inclusive Criteria from a Total of 226 Retrieved Publications

The flow chart of this review is showed in Figure 1. Three original studies on BCG vaccination contained 59 vaccinated, 45 nonvaccinated infants and 12 children²⁴ (Table I). Six original

studies on Mtb infection contained 148 patients with active TB, 121 individuals with latent TB, 111 with negative TST (Tables II, III).

BCG Induced the Production of IL-17 and IFN- γ

The level of IL-17 and IFN- γ were extremely high in the whole blood from infants or children vaccinated with BCG than those nonvaccinated with BCG while IL-4 increased slightly or maintained (Table I). PPD-stimulated whole blood cells from the vaccinated infants secreted more IL-17 and IFN- γ than the nonvaccinated in two included studies with $p < 0.0001$, while IL-4 increased in one study with $p < 0.05$ (no test in another). By the same token, the level of IL-17 as well as IFN- γ was higher in children after BCG vaccination than before ($p < 0.05$) while IL-4 kept constant ($p > 0.05$).

IL-17 and IFN- γ Had a Similar Trend in the Cases of TB Infection

As it showed in Table II, the comparisons between active or latent TB and the uninfected

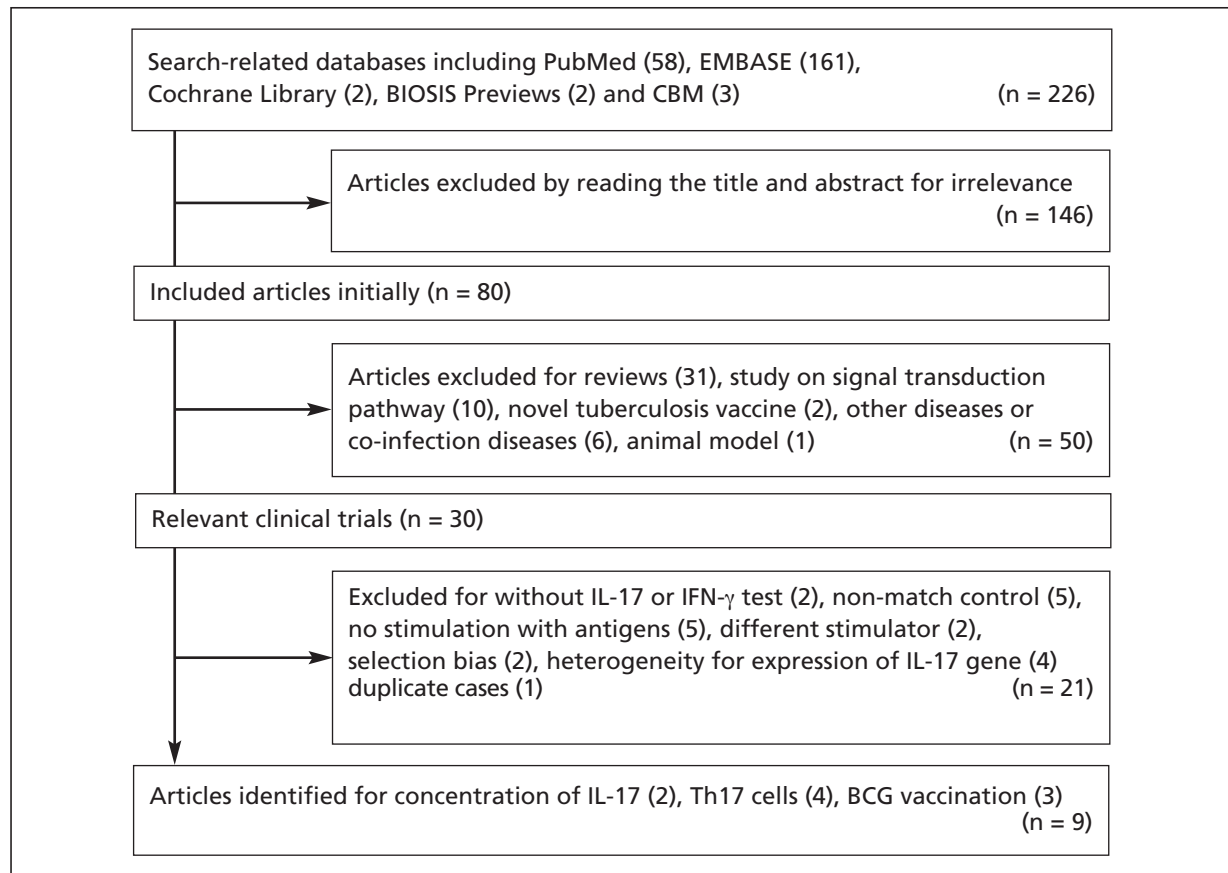


Figure 1. Flow chart of study selection.

Table I. Characteristics and comparison of included studies on BCG-induced IL-17 and IFN- γ .

Study	Number (V/N)	Vaccinated age	Stimulation (antigen/time)	Laboratory (specimen/technique)	Detected time	BCG strain	Subjects country	Results(V/N/p)		
								IL-17	IFN- γ	IL-4
Lalor 2010 ³⁶	19/9	7 w	PPD/6d	Whole blood/ELISA	3 mon	Unclear	UK	17/1.6 [#]	1705/1.6 [#]	10/1.6 [#]
Burl 2010 ³⁷	40/36	At birth	PPD/5d	Whole blood/ELISA	4.5 mon	Russian strain	Gambian	Hi/Lo [#]	Hi/Lo [#]	No test
Smith 2010 ³⁴	12 ^A	12.5 y	PPD/6d	Whole blood/ELISA	12 mon	Danish strain	UK	22.0/2.6 [#]	79.0/5.3 [#]	1.6/1.6 [*]

[#] $p < 0.0001$; ^{*} $p < 0.05$; ^{*} $p > 0.05$. V: vaccinated; N: nonvaccinated; Hi: high; Lo: low. ^AChildren (12-13 years old) are invited to take part and exclusion is based on evidence of previous BCG vaccination (BCG scar or subsequent positive tuberculin skin test). Cytokines are measured prior to and 12 months after BCG vaccination.

Table II. Characteristics and comparison of included studies on TB infection (active or latent) and negative TST.

Study	Number (Ex/Co)	Median age (Ex/Co)	Stimulation (antigen/time)	Laboratory (specimen/technique)	Subjects country	BCG vaccination	Anti-TB treatment	TB Localization	Results(Ex/Co/p)		
									IL-17	IFN- γ	IL-4
Seriba 2008 ³⁸	10(A)/22(H)	Adult/adult	PPD/12h	Whole blood/ FCA	South Africa	At birth	No	PTB	Lo/Hi [#]	Lo/Hi [#]	No test
Chen 2010 ²⁰	62(A)/66(H)	30/28	PMAl/ 6h	Whole blood/ FCA	China	Unclear	In 1 w	48 PTB 14 EPTB	Lo/Hi [#]	Lo/Hi [#]	Hi/Lo [#]
Cowan 2012 ²²	9(A)/10(H)	52/32	Mtb antigen/7d	PBMC/FCA	Canada	Unclear	No	7 PTB 2 EPTB	Lo/Hi [*]	Lo/Hi [*]	No test
Chen 2010 ²⁰	27(L)/66(H)	28/28	PMAl/6h	Whole blood/ FCA	China	Unclear	No	-	Lo/Hi [*]	Hi/Lo [#]	Hi/Lo [#]
Babu 2010 ²¹	15(L)/13(H)	32/30	PPD/24h	PBMC/ELISA	South India	At birth	No	-	105.2/823.7 [#]	608.2/418.5 [*]	Hi/Lo [*]
Cowan 2012 ²²	21(L)/10(H)	40/32	Mtb antigen/7d	PBMC/FCA	Canada	Unclear	No	-	Lo/Hi [#]	Lo/Hi [*]	No test

[#] $p < 0.001$; ^{*} $p < 0.05$; ^{*} $p > 0.05$. Ex: experiment group indicating active TB or latent TB; Co: control group indicating negative TST; A: patients with active TB; L: subjects with latent infection; H: healthy subjects with negative TST; FCA: Flow cytometric analysis; PTB: pulmonary TB; EPTB: extrapulmonary TB; PMAl: phorbol myristate acetate and ionomycin.

Table III. Characteristics and comparison of included studies on active and latent TB infection.

Study	Number (A/L)	Median age (A/L)	Stimulation (antigen/time)	Laboratory (specimen/technique)	Subjects country	BCG vaccination	Anti-TB treatment	TB Localization	Results(A/ L/p)		
									IL-17	IFN- γ	IL-4
Sutherland 2010 ³⁹	36/20	25/27	PPD/7d	Whole blood/ELISA	Gambia	Unclear	No	PTB	Hi/Lo*	2923/2043*	No test
Marin 2010 ¹⁹	31/38	42/36	PPD/48h	PBMC/ELISPOT	Colombia	Unclear	No or in 2w	30 PTB 1 EPTB	Lo/Hi*	Hi/Lo [#]	No test
Chen 2010 ²⁰	62/27	30/28	PMAII/6h	Whole blood/ FCA	China	Unclear	In 1 w	48 PTB 14 EPTB	Lo/Hi [#]	Lo/ Hi [#]	Hi/Lo*
Cowan 2012 ²²	9/21	52/40	Mtb antigen/7d	PBMC/FCA	Canada	Unclear	No	7 PTB 2 EPTB	Lo/Hi*	Lo/Hi*	No test

[#] $p < 0.05$; * $p > 0.05$.

were respectively made for IL-17, IFN- γ and IL-4. In the first three studies, IL-17 decreased in active TB ($p < 0.001$ in two studies, $p > 0.05$ in one study) as well as IFN- γ ($p < 0.05$ in two studies, $p > 0.05$ in one study) compared with the uninfected group, but IL-4 increased significantly in one study ($p < 0.001$) and was not tested in other two studies. In the last three studies, IL-17 was lower in latent TB than the uninfected ($p < 0.05$ in two studies), while IFN- γ and IL-4 were higher in two studies.

Interestingly, compared with the uninfected, IL-17 was dramatically lower in active TB ($p < 0.001$ in two studies, $p > 0.05$ in one study) while slightly lower in latent TB ($p < 0.05$ in two studies, $p > 0.05$ in one study), and IFN- γ was slightly lower ($p < 0.05$ in two studies, $p > 0.05$ in one study) in active TB while higher in latent TB. In addition, IL-17 in each study was always lower than IFN- γ whether in active or in latent TB. These information hinted there remained a same trend in IL-17 and IFN- γ during TB development.

Active and Latent TB Infection Induced IL-17 and IFN- γ Production Without Significant Difference

The production of IL-17 and IFN- γ in active and latent TB was analyzed in four studies (Table III). Although there were no significant difference for IL-17, IFN- γ and IL-4 between active and latent TB following combination of four studies, IL-17 production could be higher in latent than in active TB owing to three of four studies (with significance in one study).

Discussion

This systematic review shows that IL-17 acts as an effector molecule similar to IFN- γ after BCG vaccination and Mtb infection. IL-17 contributes to protection from TB together with IFN- γ .

BCG is proved to be effective to protect children from disseminated TB although the BCG-induced immunoprotection wanes in adults. BCG-induced Th1 immune response, which, notably secreting IFN- γ , is conventionally recognized critical to control TB, is not enough to understand the protective mechanism of BCG²⁵. In this systematic review, the increase of IL-17 was extremely analogous to IFN- γ post-BCG vaccination, which demonstrated that IL-17 besides IFN- γ was one of BCG-induced effector molecules to protect infants and children from TB.

The role for IL-17 in protection against TB analogous to IFN- γ was further demonstrated by comparison of active TB patients and the uninfected persons. IL-17 and IFN- γ levels were significantly lower in patients while IL-4 was higher. The attenuated immunoprotection response in patients might be caused by polarisation of host immunity towards a Th2 profile producing IL-4²⁶. Furthermore, the protection was also confirmed by the uptrend of IL-17 as well as IFN- γ and the downtrend of IL-4 from active to latent TB (Table II).

The adoptive transfer of IL-17-producing T cells into the IL-17KO mice, which showed an impaired mature granuloma formation and an impaired protective response to Mtb virulent strains, not only reconstituted granuloma formation which plays an essential role in the sequestration and killing of mycobacteria in the lung⁵, but also recruited neutrophils²⁷ which are likely to kill mycobacteria in case of the accumulative situations without high pathogen load or immunological dysfunction¹⁵. Like IFN- γ , IL-17 is induced during primary TB to make chemokines expressed that promote neutrophils recruitment and granuloma organization maturation throughout infection², and subsequently contributes to immunoprotection from TB^{28,29}.

There was evidence that the protective role of IL-17 was partly mediated by IFN- γ -producing Th1 cells^{30,31}. IL-17-producing Th17 cells was generated and responded more quickly than IFN- γ -producing Th1 cells to aerosol challenge in vaccinated mice. The accelerated accumulation of Th1 cells was lost along with the lack of vaccine-induced protection due to the absence of IL-17³⁰. BCG could promote the production of IL-17 that was capable to induce IL-12 and allowed the generation of subsequent Th1-cell responses. Accordingly, BCG-induced Th17-cell responses preceded the generation of Th1-cell responses *in vivo*, whereas the absence of the IL-23/IL-17 pathway decreased Th1-cell immunity and subsequent vaccine-induced protection upon Mtb challenge³¹. Therefore, these data demonstrated that IL-17/Th17 cells played an IFN- γ -dependent protective role during TB which was comparable with the findings in our systematic review.

In addition, Th1 and Th17 responses may regulate each other during Mtb infection^{17,18}. IL-12p40 is shared as a subunit by both IL-23 upon which differentiation of Th17 is dependent and IL-12p70 upon which development of Th1 responses is dependent. In the early phases of in-

fection, IL-23 is preferentially produced which promotes innate sources of IL-17 secretion. As the infection progresses, innate immune cells enhances the expression of IL-12p35, another subunit of IL-12p70, which balances IL-12p70 and IL-23 production. Meanwhile, IL-17 recruits IFN- γ -producing Th1 cells, the secreted IFN- γ inhibits Th17 development, and consequent balance between Th1 and Th17 correlates with cessation of bacterial growth^{2,5,26,32}. In other words, the protection partly requires the cooperation of IL-17 and IFN- γ .

Besides that, IL-17 provided protection in a way independent on IFN- γ . Adoptive transfer of both Th17-skewed cells and Th1-skewed T-bet deficient cells, the similarity of the two subsets is to express both IL-17 and transcription factor ROR γ t (activating IL-17 gene but restraining IFN- γ and IL-4 genes), respectively inhibited Mtb growth⁶. The reduced bacterial load in mice was associated with significant prolongation of survival following transfer of IFN- γ (-/-) Th17 cells compared to recipients of naive IFN- γ (-/-) T cells³³. In this systematic review, IL-17 production tended to be higher (high in three of four studies) in latent than active TB, outweighing IFN- γ and IL-4 as it is showed in Table III. It suggested that IL-17 might provide IFN- γ -independent protection against Mtb, which was verified by the detection of IL-17 and IFN- γ mRNA in latent and active TB. Stern et al³⁴ demonstrated that higher IL-17 mRNA was detected in PBMC of latent TB than active TB with the stimulation by PPD, while there was no significant difference for IFN- γ . Rahman et al³⁵ also indicated that lower IL-17 mRNA was detected in lymph node of children with lymph node TB than in lymphoid tissue (tonsils) from age-matched children undergoing non-infectious tonsil hyperplasia, while IFN- γ mRNA had no significant difference between the two groups. The further research on simultaneous expressions of IL-17 mRNA and protein during TB is still necessary.

Conclusions

IL-17 acts as an effector molecule similar to IFN- γ after BCG vaccination and Mtb infection. IL-17 contributes to protection against TB dependent or independent on IFN- γ . The findings do not support IL-17 as an inducer of tissue damage. Our results provide a new perspective of IL-17 for evaluation of TB vaccine and understanding of TB immunologic mechanism.

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