Systematic Review of the Non-specific Immunological Effects of Selected Routine Childhood Immunizations

APPENDIXES

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Abbreviations

BCG	Bacillus Calmette–Guérin
CD	cluster of differentiation
CI	Confidence interval
CSF	Colony-stimulating factor
CVL	Central Veterinary Laboratory
DTP	diptheria-tetanus-pertussis
EGF	Epidermal growth factor
E-Z	Edmonston Zagreb
FGF	fibroblast growth factor
Flt-3L	Flt3-ligand
GM	geometric mean
GMR	geometric mean ratio
GRO	GRO protein (cytokine)
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IPP	isopentenyl pyrophosphate
IQR	Inter-quartile range
LPS	Lipopolysaccharides
MCP	Monocyte chemoattractant protein
MDC	Monocyte depleted mononuclear cells
MFI	Mean fluorescence index
MIP	Macrophage inflammatory protein
MMR	Measles mumps and rubella
NSIE	Non-specific immunological effects
PBMC	Peripheral blood mononucleated cell
PDGF	Platelet-derived growth factor
pg	Picograms
PHA	Phytohaemagglutinin
PMA	phorbol myristate acetate
PPD	Purified protein derivitive
RCT	Randomized controlled trial
RPMI	Roswell Park Memorial Institute medium
SAGE	Strategic advisory group of experts
SEB	Staphylococcal enterotoxin B
SK/SD	Streptokinase/Streptodornase
SSI	Staten Serum Institut
TLR	Toll-like receptors
TNF	Tumor necrosis factor
TT	Tetanus toxoid
UCL	University College London
UK	United Kingdom

VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Executive Summary

There is clear scientific evidence that exposure to infectious agents and vaccines results in non-specific inflammatory and innate immunological responses that subsequently direct "acquired" specific immunity through T cells and antibody, that recognise antigenic epitopes on the organism or vaccine. This concept underpins modern immunology and vaccinology. Agents, including infectious diseases, which modify these early non-specific signals might have important effects on the response that develop to a subsequent heterogenous stimulus. Despite widespread acceptance amongst immunologists that such non-specific immunological effects occur, there are no systematic studies which have summarised the literature in humans to provide a framework for understanding the circumstances under which such effects can be documented, when such responses occur, or for how long they are present or, most importantly, their biological significance.

In this review, conducted at the request of WHO, we have systematically searched the scientific literature to identify available data concerning non-specific effects which might be measured after immunisation with the main vaccine antigens that are including in the expanded programme of immunisation for children, namely, BCG, measles, diphtheria, tetanus and pertussis.

The review demonstrates that there are a substantial number of studies which contain data of relevance to the assessment, but the vast majority were not conducted to investigate this phenomenon, though they did report data which could be extracted. There were few studies with similar methodology or endpoints which could be formally meta-analysed and therefore data are presented in summary figures and tables for each vaccine. The reviewed studies were highly heterogeneous and the risk of bias was high or unclear in the majority. While some significant findings were present, the lack of replication of the findings and the low quality of the majority of studies, indicates that such findings should be interpreted with caution.

While these findings do not exclude the possibility of important non-specific immunological effects of vaccines, the published literature dose not provide confidence in the presence, quality, quantity, kinetics or impact of any non-specific immunological effects in young children after vaccination. It is, therefore, not currently possible to provide any guidance from the human data on expected effects or when/how to measure them.

Future studies using systems biology to capture the functional genomic, genetic, epigenetic and immunological effects of vaccines, might be applied to explore this biological phenomenon and to provide data on the timing, duration, quality and magnitude of such effects and to identify signals which might be used in large scale studies with relevant epidemiological endpoints.

Background

A growing number of published reports have suggested that several vaccines routinely administered to infants around the world may have "heterologous" or "non-specific" effects on mortality unrelated to prevention of illness and deaths caused by the specific diseases against which the vaccines have been formulated. For example, studies have suggested that receipt of both the Bacillus Calmette–Guérin (BCG) and measles vaccine are associated with a reduced risk of death (i.e. all cause mortality), while receipt of diphtheria-tetanus-pertussis (DTP) vaccine is associated with an increased risk of death, at least among female infants.[1,2] The vast majority of the studies demonstrating these effects have been observational in nature, rather than randomised controlled trials with non-specific effects as the primary outcome, and as a result, poorly-controlled or uncontrolled confounding and various types of selection and information bias have been suggested as alternative explanations for these findings.[3,4]

The biological plausibility of one or more vaccines having heterologous effects, either detrimental or beneficial, is supported by a number of studies in animals (for example mice) and observations in humans.[5-8] Nevertheless, the biological mechanisms and immune pathways that would underlie and explain such effects remain largely unspecified and open to question. At the same time, the possible implications of any such heterologous vaccine effects for the formulation or re-formulation of the infant immunization schedule remain unclear, but it has been suggested that if such effects can be established beyond a reasonable doubt, the infant immunization schedule might need to be re-configured.[9] However, prior reviews of this subject, including periodic assessments by the World Health Organization (WHO) Global Advisory Committee on Vaccine Safety, have concluded that any such effects remain unproven and are therefore not a justification for altering the current schedule.[10]

The WHO Strategic Advisory Group of Experts (SAGE) has requested the WHO Secretariat to review the evidence surrounding the possible non-specific/heterologous effects of vaccines included in the routine infant immunization schedule.[9] Overall, our aim is to determine whether the current evidence is sufficiently sound to warrant further scientific investigation; and if so, to define the path towards obtaining unequivocal evidence on these issues that would support future robust, evidence-based adjustments in immunization policies, if

warranted. Preparatory to such a review of the evidence by SAGE at its April, 2013 meeting, it is necessary to assemble the available evidence, both published and unpublished, and subject that evidence to a systematic review.

Thus the objective of this review is to systematically identify, assemble, review and critically appraise all available studies with immunological endpoints describing the possible non-specific or heterologous effects of BCG, diphtheria, pertussis, tetanus and measles containing vaccines.

Appendix A: Search strategy

Embase search

Activins OR 'cytokine'/exp OR activin OR ‘adipocytokines’ OR ‘ adipokine’ OR ‘ adipokines’ OR ‘ adipose tissue derived cytokine’ OR ‘Acrp 30’ OR ‘ Acrp30’ OR ‘ adipocyte complement related protein 30’ OR ‘ adipocyte most abundant protein 1’ OR ‘ adipoq’ OR ‘ APM 1’ OR ‘ APM1’ OR ‘ GBP 28’ OR ‘ GBP28’ OR ‘ gelatin binding protein 28’ OR ‘AIF 1’ OR ‘ AIF1’ OR ‘ cytokine AIF 1’ OR ‘ cytokine AIF1’ OR ‘ daintain’ OR ‘a proliferation inducing ligand’ OR ‘ a proliferation inducing ligand protein’ OR ‘ antigen CD256’ OR ‘ CD256 antigen’ OR ‘ protein APRIL’ OR ‘ protein TALL2’ OR ‘ protein TNFSF 13’ OR ‘ protein TNFSF13’ OR ‘ TALL 2 protein’ OR ‘ TALL2 protein’ OR ‘ TNF and ApoL related leukocyte expressed ligand 2’ OR ‘ TNF related death ligand 1’ OR ‘ TNFSF 13 protein’ OR ‘ TNFSF13 protein’ OR ‘ tumor necrosis factor and apolipoprotein related leukocyte expressed ligand 2’ OR ‘ tumor necrosis factor ligand superfamily member 13’ OR ‘ tumor necrosis factor related death ligand 1’ OR ‘ tumor necrosis factor SF13’ OR ‘ tumor necrosis factor superfamily member 13’ OR ‘B lymphocyte activating factor [134-285]’ OR ‘ B lymphocyte stimulator [134-285]’ OR ‘ATX protein’ OR ‘ ectonucleotide pyrophosphatase phosphodiesterase 2’ OR ‘ ENPP2 protein’ OR ‘ PDNP2 protein’ OR ‘ protein ATX’ OR ‘ protein ENPP2’ OR ‘ protein PDNP2’ OR ‘antigen CD257’ OR ‘ B-cell activating factor’ OR ‘ B cell activation factor’ OR ‘ B lymphocyte activating factor’ OR ‘ B lymphocyte stimulator’ OR ‘ B lymphocyte stimulator protein’ OR ‘ BAFF’ OR ‘ BLyS protein’ OR ‘ CD257 antigen’ OR ‘ protein BLyS’ OR ‘ protein TALL 1’ OR ‘ protein TALL1’ OR ‘ protein TNFSF13B’ OR ‘ TALL 1 protein’ OR ‘ TALL1 protein’ OR ‘ TNF and ApoL related leukocyte expressed ligand 1’ OR ‘ TNFSF13B protein’ OR ‘ tumor necrosis factor and apolipoprotein related leukocyte expressed ligand 1’ OR ‘ tumor necrosis factor ligand superfamily member 13B’ OR ‘B cell differentiation factor’ OR ‘ bcdf’ OR ‘B cell growth factor’ OR ‘ bcgf’ OR ‘ growth factor, b cell’ OR ‘bone morphogenetic proteins’ OR ‘ bone morphogenic protein’ OR ‘BMP 12’ OR ‘ BMP12’ OR ‘ cartilage derived morphogenetic protein 3’ OR ‘ CDMP 3’ OR ‘ CDMP3’ OR ‘ GDF 7’ OR ‘ GDF7’ OR ‘ growth and differentiation factor 7’ OR ‘ growth differentiation factor 7’ OR ‘BMP 15’ OR ‘ BMP15’ OR ‘ GDF 9B’ OR ‘ GDF9B’ OR ‘ growth and differentiation factor 9B’ OR ‘ growth differentiation factor 9B’ OR ‘BMP 2’ OR ‘ BMP2’ OR ‘BMP 4’ OR ‘ BMP4’ OR ‘BMP 5’ OR ‘ BMP5’ OR ‘BMP 6’ OR ‘ BMP6’ OR ‘BMP 9’ OR ‘ BMP9’ OR ‘ GDF 2’ OR ‘ GDF2’ OR ‘ growth and differentiation factor 2’ OR ‘ growth differentiation factor 2’

OR ‘4-1BB ligand’ OR ‘ 4 1BB ligand’ OR ‘ 4 1BBL protein’ OR ‘ CD137L’ OR ‘ ligand 4 1BB’ OR ‘ protein 4 1BBL’ OR ‘antigen CD153’ OR ‘ CD153 antigen’ OR ‘ CD153 antigens’ OR ‘ CD30L’ OR ‘ protein TNFSF 8’ OR ‘ protein TNFSF8’ OR ‘ TNFSF 8 protein’ OR ‘ TNFSF8 protein’ OR ‘ tumor necrosis factor ligand superfamily member 8’ OR ‘ tumor necrosis factor superfamily member 8’ OR ‘antigen CD154’ OR ‘ CD154 antigen’ OR ‘ CD40L’ OR ‘ CD40L antigen’ OR ‘ protein TNFSF 5’ OR ‘ protein TNFSF5’ OR ‘ TNFSF 5 protein’ OR ‘ TNFSF5 protein’ OR ‘ tumor necrosis factor ligand superfamily member 5’ OR ‘ tumor necrosis factor superfamily member 5’ OR ‘antigen cd70’ OR ‘ antigens, CD70’ OR ‘ CD27 ligand’ OR ‘ CD27L’ OR ‘ CD70 antigens’ OR ‘colony-stimulating factors’ OR ‘ colony stimulating activity’ OR ‘ colony stimulating factors’ OR ‘fibroblast derived differentiation inducing factor’ OR ‘ectodermal dysplasia protein’ OR ‘ ectodysplasin 1’ OR ‘ ectodysplasins’ OR ‘ EDA A protein’ OR ‘ EDA protein’ OR ‘ protein EDA’ OR ‘ protein EDA A’ OR ‘am 424’ OR ‘ am424’ OR ‘ recombinant human leukemia inhibitory factor’ OR ‘ recombinant leukemia inhibitory factor’ OR ‘EMAP II’ OR ‘ endothelial monocyte activating polypeptide 2’ OR ‘antigen CD178’ OR ‘ CD178 antigen’ OR ‘ CD95 ligand’ OR ‘ CD95L’ OR ‘ CD95L protein’ OR ‘ Fas antigen ligand’ OR ‘ Fas ligand protein’ OR ‘ FasL protein’ OR ‘ protein CD95L’ OR ‘ protein FasL’ OR ‘ protein TNFSF 6’ OR ‘ protein TNFSF6’ OR ‘ TNF superfamily member 6’ OR ‘ TNFSF 6 protein’ OR ‘ TNFSF6 protein’ OR ‘ tumor necrosis factor ligand superfamily member 6’ OR ‘fibroblast growth factors’ OR ‘ fibroblast stimulating factor’ OR ‘ heparin binding growth factor’ OR ‘fgf 1’ OR ‘ FGF1’ OR ‘fgf 10’ OR ‘ FGF10’ OR ‘ fgf 14’ OR ‘ FGF14’ OR ‘ fgf 16’ OR ‘ FGF16’ OR ‘ fgf 18’ OR ‘ FGF18’ OR ‘ fgf 19’ OR ‘ FGF19’ OR ‘ fgf2’ OR ‘ FGF 2’ OR ‘ fgf21’ OR ‘ FGF 21’ OR ‘ fgf23’ OR ‘ FGF 23’ OR ‘ fgf3’ OR ‘ FGF 3’ OR ‘ fgf4’ OR ‘ FGF 4’ OR ‘ fgf5’ OR ‘ FGF 5’ OR ‘ fgf6’ OR ‘ FGF 6’ OR ‘ fgf8’ OR ‘ FGF 8’ OR ‘ fgf9’ OR ‘ FGF 9’ OR ‘interleukin’ OR ‘interleukins’ OR ‘il 1’ OR ‘il 2’ OR ‘il 4’ OR ‘il 5’ OR ‘il 6’ OR ‘il 9’ OR ‘il 10’ OR ‘il 12’ OR ‘il 13’ OR ‘il 17’ OR ‘il 23’ OR ‘interferon’ OR ‘helper cell type 1’ OR ‘ T helper 1’ OR ‘ T helper type 1’ OR ‘ Th1 cells’ OR ‘helper cell type 2’ OR ‘ T helper 2’ OR ‘ T helper type 2’ OR ‘ Th2 cells’ OR 'helper cell'/exp OR 't helper' OR 'B lymphocyte'/exp OR ‘B-lymphocyte subsets’ OR ‘ B-lymphocytes’ OR ‘ b-lymphocytes, regulatory’ OR ‘ B cell’ OR ‘ bone marrow derived lymphocyte’ OR ‘ bone marrow lymphocyte’ OR ‘ bursa derived lymphocyte’ OR ‘ lymphocyte, b’ OR ‘ lymphocyte, bone marrow derived’ OR ‘ lymphocyte, bursa derived’ OR ‘ regulatory B lymphocyte’ OR ‘antibody-producing cells’ OR ‘ antibody forming cell’ OR ‘ antibody producing cell’ OR ‘ immunoglobulin forming cell’ OR ‘B memory cell’ OR ‘ B memory cells’ OR ‘ B memory

lymphocyte’ OR ‘ B memory lymphocytes’ OR ‘ memory B cell’ OR ‘ memory B cells’ OR ‘ memory B lymphocyte’ OR ‘ memory B lymphocytes’ OR ‘cell, plasma’ OR ‘ flamed plasma cell’ OR ‘ flamed plasmacell’ OR ‘ plasma cells’ OR ‘ plasmacyte’ OR ‘ plasmatocyte’ OR ‘ plasmocyte’ OR ‘ plasmocyte, flamed’ OR ‘B cell precursor’ OR ‘ B cell precursors’ OR ‘ B cell progenitor’ OR ‘ B cell progenitors’ OR ‘ B lineage precursor’ OR ‘ B lineage precursors’ OR ‘ B lineage progenitor’ OR ‘ B lineage progenitors’ OR ‘ B lymphocyte precursor’ OR ‘ B lymphocyte progenitor’ OR ‘ B lymphocyte progenitors’ OR ‘ B lymphoid precursor cell’ OR ‘ B lymphoid precursor cells’ OR ‘ B lymphoid progenitor’ OR ‘ B lymphoid progenitors’ OR ‘ B precursor’ OR ‘ B precursors’ OR ‘ B progenitor’ OR ‘ B progenitors’ OR ‘ cell, pre B’ OR ‘ immature B cell’ OR ‘ immature B cells’ OR ‘ pre B cell’ OR ‘ pre B cells’ OR ‘ precursor B cell’ OR ‘ precursor B cells’ OR ‘ precursor B lymphocyte’ OR ‘ precursor B lymphocytes’ OR ‘ precursor cells, B-lymphoid’ OR ‘ precursor cells, B lymphoid’ OR ‘ pro-B cell’ OR ‘ pro-B cells’ OR ‘ progenitor B cell’ OR ‘ progenitor B cells’ OR ‘ transitional B cell’ OR ‘ transitional B cells’ OR " tumor necrosis factor receptor 1"/exp OR " tumor necrosis factor receptor 1 " OR " CD120a antigen" OR " receptors, tumor necrosis factor, type I" OR " tumor necrosis factor receptor type 1" OR " tumor necrosis factor receptor type I" OR 'dendritic cell'/exp OR ‘dendritic cells’ OR ‘dendritic cell’ OR ‘langerhans cell' OR 'langerhans cells' OR 'T lymphocyte'/exp OR ‘amplifier t lymphocyte’ OR ‘ lymphocyte, thymus’ OR ‘ T-lymphocytes’ OR ‘ t-lymphocytes, suppressor-inducer’ OR ‘ T cell’ OR ‘ T cells’ OR ‘ thymic lymphocyte’ OR ‘ thymus dependant lymphocyte’ OR ‘ thymus dependent cell’ OR ‘ thymus dependent lymphocyte’ OR ‘ thymus derived cell’ OR ‘ thymus derived lymphocyte’ OR ‘ thymus lymphocyte’

This in combination with - Any Bacillus Calmette Guerin containing vaccine - Any Diphtheria toxoid containing vaccine - Any Tetanus toxoid containing vaccine - Any *Bordetella pertussis* containing vaccine - Any measles containing vaccine

PubMed search

((((("T-Lymphocytes, Helper-Inducer"[Mesh]) OR "T-Lymphocytes, Regulatory"[Mesh]) OR "Cytokines"[Mesh]) OR "Antigen-Presenting Cells"[Mesh]) AND ((("BCG Vaccine"[Mesh]) OR "Measles Vaccine"[Mesh]) OR "DTP Vaccine"[Mesh])

Cochrane search

((((("T-Lymphocytes, Helper-Inducer"[Mesh]) OR "T-Lymphocytes, Regulatory"[Mesh]) OR "Cytokines"[Mesh]) OR "Antigen-Presenting Cells"[Mesh]) AND ((("BCG Vaccine"[Mesh]) OR "Measles Vaccine"[Mesh]) OR "DTP Vaccine"[Mesh])

From this search only trials are going to be included.

Trip search

BCG vaccine OR Measles vaccine OR DTP vaccine

Appendix B: Description of included studies

BCG

Author	Methods	Participants	Interventions	Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Akkoc *et al* 2010	Open, randomized, single centre trial	Healthy term newborns (n=19)	BCG vaccination at birth (n=10) compared to BCG vaccination at 2 months (n=9)	Cytokine responses	IFN-γ and IL-10 responses to PBMCs stimulated with PHA	pg/ml	NS between groups.	
Anderson *et al* 2013	Randomised, control trial, Samples at 6-11 weeks (early sample group, n=124) or5-9 months (late sample group, n=221).	Infants in Guinea-Bissau, n=357 (345 included in analysis)	BCG revaccination at 19 months, n=158 or nothing, n=187	IFN-γ, IL-13, TNF- α and IL-10 stimulated with LPS, PPD, or PHA.	IFN-γ, IL-13, TNF- α and IL-10 stimulated with LPS or PHA.	Geometric mean/geometric mean ratio	NS for IFN-γ and IL-13	Part of the REVAC trial which was halted prematurely due to excess mortality.
Black *et al* 2001	Randomised, controlled trial, single region	Healthy individuals from Northern Malawi with no prior history of BCG vaccination	BCG compared to placebo (dextran matrix of BCG vaccine), single dose.	Skin tests and IFN-γ responses to Mycobacterial spp.	IFN-γ responses to control samples in lymphocyte cultures	pg/ml	NS	Study carried out in the context of the Karonga Prevention Study, a large vaccine trial and epidemiological study of tuberculosis and leprosy in the Karonga district.
Black *et al* 2002	Randomised, controlled	Healthy young adults from	BCG vaccination compared to	IFN-γ responses to control antigens	IFN-γ responses to control antigens	pg/ml	NS	Study carried out in the context of

	trial, multiple regions	Northern Malawi (n= 633, mean age 19 years) and East London and Essex, United Kingdom (n=424, mean age 14 years)	placebo	and PPD, and skin testing to mycobacterial antigens				the Karonga Prevention Study, a large vaccine trial and epidemiological study of tuberculosis and leprosy in the Karonga district.
Burl *et al* Jul. 2010	Open, randomised, single centre trial	Healthy Gambian newborns (n=103)	BCG vaccination at birth (n=53) compared to BCG vaccination at 4.5 months (n=50)	Cytokine responses and cell phenotyping	Whole blood phenotyping IFN-γ, IL-13, IL-6, IL-17 and IL-10 production by whole blood stimulated with control antigens	% CD4+CD25+ T cells and % CD4+CD25+FOXP3+ T cells Log10pg/ml	NS	
Burl *et al* Aug. 2010	Open, randomised, single centre trial	Healthy Gambian newborns (n=103)	BCG vaccination at birth compared to BCG vaccination at 4.5 months	Immune responses to tuberculin skin tests, cytokine studies and cellular phenotyping	Controls (SEB) used for whole blood IFN-γ and IL-10 responses (not reported) T cell phenotype	pg/ml %CD4+CD25+ T cells and %CD4+CD25+FOX3 + T cells	Non-specific data not reported therefore significance unknown	
Djuardi *et al* 2010	Birth cohort from two regions in Indonesia	Newborns (n=147, mean age at vaccination 5 weeks, IQR 2-8.5)	Infant vaccination program comprising, BCG, Hepatitis B, DTP, OPV and measles	Cytokine responses	IFN-γ, IL-5, TNF-α, IL-10 and IL-13 responses from whole blood cultures stimulated	pg/ml and median with IQR	Sig increase in IFN-γ production, $p<0.001$, NS for IL-5 and IL-13 (to PHA from pre	

			vaccines.		with PHA, LPS and media only		vaccination to 2 years). Sig decrease for TNF-α and IL-10 (LPS)P<0.001.	
Elliot *et al* 2011 (BCG and TT)	Observational analysis of newborns after a randomised, double blinded, controlled trial of anti-helminth therapy in pregnant women	Newborns of women recruited from a Ugandan hospital antenatal clinic (n=2345).	Infant vaccination program comprising of BCG, polio, diphtheria, pertussis, tetanus, hepatitis B, *H. influenzae* and measles vaccines.	Cytokine responses to BCG and TT	IFN-γ, IL-5, IL-13 and IL-10 responses to control stimulated cultures (not reported)	Geometric mean ratios	Not specific results/significance not reported	
Faustman *et al* 2012	Double blinded, randomised, controlled, single centre, trial	Long term type 1 diabetic adults (n=6) and healthy non-diabetic controls	BCG vaccination (n=3 diabetics and n=3 controls) compared to placebo (n= 3 diabetics and n=3 controls)	T-cells, auto-antibodies and C-peptide	Autoreactive T cells Insulin autoantibodies (GAD, IA-2A, ZnT8A) C-peptide	% Units pmol/L	Autoreactive T cells -Significance tests not reported Insulin autoantibodies-GAD:2 BCG treated subjects significant changes from baseline (one increase, one decrease p=0.0001/0.0017). ZnT8A: sig decrease in one BCG subject only	

							Transient and significant rise in C-peptide level in BCG subjects	
Fjallbrant *et al* 2007	Cohort study, all subjects immunised and blood taken before and 2 months and 1 year after vaccination.	TST negative, healthy students in Sweden either never vaccinated (n=15) or previously vaccinated (n=16), total n=31.	BCG vaccine	Lymphocyte transformation stimulated with PPD, ConA and unstimulated. Cytokine levels from supernatants, stimulated as above	Lymphocyte transformation stimulated with ConA and unstimulated. Unstimulated and ConA stimulated cytokine levels	CPM	Non-specific data not reported	
Gruber *et al* 2000	Prospective cohort of vaccinated and unvaccinated children. Samples taken at birth, 12, 24, 36, 60, 72 and 84 months.	Part of a neonatal birth cohort in Germany, (MAS-90 study group), total n=1314. Included in this analysis, n=774	Children at high risk for TB were vaccinated with BCG,median age 30days (range 1-343) n=169. Included in this analysis, n=92.	Atopic manifestations Total and specific IgE	Atopic manifestations Total and specific IgE	% with specified symptoms kU/L	NS NS (NB percentage of positive IgE tests from total tests performed was lower in BCG groups in first 3 years then higher at 5 and 7 years)	
Hoft *et al* 1998	Double-blinded, randomized controlled study	Healthy adults (n=54, age 18-45 years)	Connaught BCG (n=18), tice BCG (n=18), placebo (n=18)	T cell proliferation to mycobacterial antigens and controls, T cell subset expansion proliferation to mycobacterial antigens and	T cell proliferation responses to media and tetanus toxoid T cell subset expansion to media and tetanus toxoid	fold increase dpm of day 56 compared to day 0 mean % of cells	NS NS	

				controls, T cell subsets following IL-2 and IPP stimulation	T cell subsets following IL-2 and IPP stimulation	Absolute number of CD4+, CD8+ and γδ T cells	Sig increase in BCG responders (P<0.01, 0.02, 0.03)	
Hoft *et al* 1999	Double-blinded, randomized controlled study and an open label study	Healthy adults (n=66, age 18-45 years)	Connaught BCG (n=12, open label, n=18 double blind), Tice BCG (n=18 double blind), placebo (n=18 double-blind)	T cell proliferation, IFN-γ and IL-4 production, cytotoxicity assays, and mycobacterial antibodies	T cell proliferation to PHA and media alone IFN-γ and IL-4 ELISpot responses from PBMCs incubated with media alone IFN-γ and IL-4 ELISAs of culture supernatants of PBMCs with media alone	dpm spot forming cells/1^4 cells pg/ml	Non-specific data not reported	
Hussey *et al* 2002	Five groups with different vaccine timing and methodologies	Healthy newborns recruited from primary care services	Danish BCG at birth intradermally n=11, Danish BCG at 10 weeks intradermally n=11, Japanese BCG at birth intradermally n=10, Japanese BCG at birth percutaneously using Bignell tool	PBMC proliferation, cytokine responses and cytotoxicity	PHA and TT proliferation responses TT IFN-γ, IL-10 and IL-5 responses	pg/ml pg/ml	NS NS	

			n=10, Japanese BCG at birth percutaneously using Biovac tool n=20.					
Kagina *et al* 2009	Single centre, randomized study	Healthy newborns (n=46)	BCG vaccination at birth (n=25) or delayed until 10 weeks of age (n=21)	Whole blood intracellular cytokines (IFN-γ, TNF-α and IL-2), and Lymphocyte phenotyping of BCG specific cells	Whole blood intracellular cytokines (IFN-γ, TNF-α and IL-2)	% of cytokine positive CD4+ T cells	NS (in supplementary data)	
Kleinnijenhuis *et al* 2012	Open label cohort study	Healthy adults aged 20-36 years scheduled to receive BCG due to travel to TB endemic regions (n=20).	BCG vaccination	Cytokines in response to TB, *S. aureus* and *C. albicans* and phenotype of circulating monocytes.	IFN-γ, TNF-α and IL-1β production from PBMC cultures stimulated with *S. aureus* and *C. albicans* CD14+ cells TLR4 and CD11b surface expression IL-1β and TNF-α mRNA expression	fold induction % of total MFI Fold induction	Sig increase from baseline at 2w and 3m (except *S. aureus* and IL-1β at 2 weeks), p<0.05 or 0.01 Sig increase at 2 weeks (p<0.05) TLR4: Sig decrease at 2 weeks (p<0.05) and increase at 3m (p<0.005) CD11b sig increase NS	
Lalor *et al* 2009	Combination of a case-control study in the UK and a cohort in Malawi	Healthy infants from the UK (n=117) and Malawi (n=615)	UK - BCG vaccination compared to unvaccinated age-match controls Malawi – BCG	IFN- γ responses to PPD	IFN-γ responses from whole blood cultures stimulated with control antigens	pg/ml	Statistical comparisons not reported	

			vaccination					
Lalor *et al* 2010	Case-control study comparing vaccinated and unvaccinated infants 3 months after vaccination.	UK infants, n=28	Cases: BCG vaccination at 5-10 weeks (mean 7 weeks) Controls: no BCG.	IFN-γ production stimulated a by PHA, M.tb PPD or unstimualted. 21 cytokine and chemokines in supernatants stimulated by M.tb PPD or unstimulated	IFN-γ production stimulated by PHA, or unstimualted. 21 cytokines and chemokines in unstimulated supernatants.	Pg/ml	Unstimulated control data not reported.	Infants had previously taken part in an IFN ga,,a study
Lalor *et al* 2011	Sub-group of a larger study consisting of a combination of a case-control study in the UK and a cohort in Malawi	Healthy infants from the UK (n=28 vaccinated and n=9 unvaccinated) and from Malawi (n=40 vaccinated)	UK - BCG vaccination compared to unvaccinated age-match controls Malawi – BCG vaccination	Cytokine, chemokine and growth factor responses.	Large panel of cytokine and chemokine responses (multiplex bead array) from whole blood cultures stimulated with control antigens	pg/ml	Control data not reported	
Libraty *et al* 2014	Case-control study (nested into a dengue virus study)	Infants who had not received BCG in the first 2 weeks of life (n=13) and age- and sex match infants who did receive BCG in the first two weeks (n=38)	BCG in first two weeks or delayed until after first vaccination with DTP	IFN-γ ELISpot. Flow cytometry	IFN-γ ELISpot to TT to Polio to HBVsAg to PHA Tcells -TNF-α/CD4+/CD4RO+ Tcells - TNF-α/CD4+/CD4RO-	SFC/10^6 cells SFC/10^6 cells SFC/10^6 cells % cells % cells	p=0.046, NS p=0.4 NS p=0.018	

					Tcells -FoxP3+TNF-α/CD4+/CD4RO+ Tcells -FoxP3+TNF-α/CD4+/CD4RO-	% cells % cells	NS NS NS	
Lowry *et al* 1998	Randomised, placebo-controlled trial comparing different doses of BCG with saline. Blood samples before, 2 and 16 weeks and 1 year after vaccination.	Healthy adults aged 18-44 years born in the USA, not previously vaccinated with BCG, n=76	Very low dose BCG, low dose BCG, standard dose BCG or high dose BCG or saline placebo. 5 subjects from very low, low and placebo groups were revaccinated at 18 weeks.	Adverse events. PPD skin tests. Lymphopriliferation to M TB, Ag85, BCG, *M avium* and ConA. IL-4 and IFN-γ stimulated as above IgG seroractivity to BCG-SA antigen	Lymphoprolieration to Con A IL-4 and IFN-γ to ConA (not reported)	CPM Not reported	NS (p value not reported) Not reported	
Marchant *et al* 1999	Single centre, randomised, controlled trial	Healthy newborns (n=137)	BCG vaccination given at birth, 2 months or 4 months.	Whole blood and PBMC proliferation and cytokine responses	lymphoproliferation IFN-γ, IL-5, IL-13 and IL-4 responses following culture with PHA	Stimulation index pg/ml	NS NS	
Marks *et al* 2003	Retrospective case-control study across two regions of Sydney, Australia	Children from a region where BCG vaccination was routine (n=309, age 9.5+/-2.4 years) and another region	BCG vaccination compared to unvaccinated	Cytokine responses, Clinical components of allergy	IL-4, IL-5, IL-10 and IFN-γ secreted in response to house dust mite stimulation of PBMCs	Stimulation index, median with bow and whisker plots Geometric mean	IL-10 SI significantly lower for BCG recipients, p<0.0001. NS for other cytokines	

		where it was not (n=442, age 9.7+/-2.0).			Serum total IgE	kIU/L	Comparison not reported	
Miles *et al* 2008	Cohort study, all infants vaccinated and part of a larger CMV cohort study.	Male and females infants in the Gambia taking part in a CMV study, all infected with CMV, n=133 (though not all sampled at all time-points). Children aged 4-5 years in the Gambia, n=25 and adult females aged 21-31 years who had given birth 18 months ago , n=11.	Infants were immunised at birth with BCG. All children were recorded to have received BCG. Adults assumed to have had BCG.	IFN-γ, IL-2, CD154 responses of CD4 T cells stimulated with PPD or normal human dermal fibroblast lysate (NHDF as required by CMV study)	IFN-γ IL-2, CD154 responses of CD4 T cells stimulated NHDF (not reported).	% of CD4 cells expressing the cytokine, median with interquartile range.	Not reported	Part of a larger CMV study and all infants had CMV infection.
Miles *et al* 2009	Single centre, case-control study examining maternal HIV status on newborns response to BCG	Newborns of HIV positive (n=16) and negative (n=21) Malawian women	BCG vaccination at birth (OPV also given).	IFN- γ responses to BCG and PPD, cellular phenotypes. PHA controls.	CD4 counts in HIV negative infants born to HIV negative mothers CCR7 and CD45RA expression CD27 and CD28 expression PD-1+ and CD57+, CD4+ T cells IFN-γ release by PBMC s	Median with IQR Cells/ml blood x 10^5 Cells/ml blood x 10^5 % Spot forming units per 10^6 PBMCs	Significance reported between groups (based on HIV status of mother), all of whom were vaccinated.	

Ota *et al* 2002	Single centre, randomised trial	Healthy newborns (n=151)	BCG vaccination given at birth, 2 months or 4.5 months. Routine vaccine schedule (OPV, DTP and Hepatitis B vaccine) also given	Proliferation, cytokine and vaccine specific antibody responses	PBMC proliferation to HBsAg, TT and PHA IL-5, IL-13 and IFN-γ PBMC responses to HBsAg, TT and PHA Antibody responses to HBsAg, TT, PV1, DT	Stimulation index pg/ml mIU/ml	Significant between groups Significant between groups Significant between groups	
Smith *et al* 2012	Two case-control studies:- one in infants the other in adolescents	UK born BCG vaccinated infants (n=21 mean age 11.5, SD 6.8 weeks) and unvaccinated (n=18, mean age 13, SD 3.4). Adolescents vaccinated with BCG (n=16) and unvaccinated (n=20)	BCG vaccinated compared to unvaccinated	Cytokine and chemokine responses to PPD and Heparin-binding haemagglutinin	PHA used as positive control (reported in supplemental data).	Pg/ml Median with interquartile range	NS	
Soares *et al* 2013	Cohort study	Healthy newborns (n=90)	BCG vaccination at birth	Intracellular cytokine expression of T cells.	PMA, TT, PHA stimulated cytokine responses	Data not reported		
Steenhuis *et al* 2007	Randomized, prospective, single blinded (researcher) trial to determine effect of BCG	Mainly Caucasian newborns with mother or father and sibling with allergic disease (n=121)	BCG vaccination or placebo (normal saline) at 6 weeks of age	Prevalence of allergic disease at 18months.	Prevalence of allergic disease at 4 and 18months. Leucocytes and eosinophils in peripheral blood	Questionnaire, SCORAD scoring, lung function Mean leucocytes and eosinophils ±SD	NS	19 participants had BCG repeated at 4 months as no scar and negative skin test.

	on allergic disease.							
Tastan *et al* 2005	Randomised trial comparing vaccination at birth or 2 months. Blood taken at 48 hours (group 1 only) 2 months and 4 months (group 2 only) of age.	Healthy, male and female, term newborns in Turkey, n=40.	Group 1: BCG vaccine at birth, n=20 Group 2: BCG vaccine at 2 months, n=20 NB all received routine immunisations including DTP and polio at 2, 4 and 6 months	Total lymphocytes	Total lymphocytes- TCR+ and TCR-. TCR+ cells- αβ+ and γδ+	Cells/µl, median value with quartiles.	Significant different between groups at 2months for total lymphoctyes, TCR- (increased in vaccinated) and αβ+ cells (decreased in vaccinated).	
Van den Biggelaar *et al* 2009	Case-control	Healthy newborns from PNG (n=50) and Australia (n=50). Healthy Australian adults as controls (n=15)	BCG vaccination in PNG newborns. No BCG vaccination in Australian participants	Mononuclear cell culture cytokine production. mRNA expression. Lymphocyte phenotyping	Cytokine (IL-10, IFN-γ, TNF-α, IL-5) responses to PHA	Data not reported		
Vargas *et al* 2004	Randomised, placebo controlled trial investigating effect of BCG on asthma. Samples taken before and 12 months after vaccination.	Asthmatic schoolchildren, male and female, in Mexico, n=82 (67 completed study)	BCG, n=33 or placebo (saline), n=34. NB all children were treated for their asthma for 12 months prior to vaccination	Symptoms questionnaire Leukocyte count, eosinophil count, IgE, esosinophils in nasal mucus and parasites in stools. Subset (17 vaccinated and 18 placebo) had IL-4 and IFN-γ from phorbol myristate acetate and ionomycin stimulated PBMCs	Symptoms questionnaire Leukocyte count, eosinophil count, IgE, esosinophils in nasal mucus and parasites in stools. IL-4 and IFN-γ from phorbol myristate acetate and ionomycin stimulated PBMCs.	Cells/mm3, IU/ml Pg/ml	Within group sig change over time for controls but not for vaccinated for IgE, IL-4 and IFN-γ. Within group sig decrease in monocytes, eosinophiles and % eosinophils in nasal cytology for BCG. Between group comparisons not	

							done.	
Vekemans *et al* 2004	Single centre randomized study in newborns and a case-control study in adults	Healthy newborns (n= 29) Adults with tuberculosis (n=33), TB exposed (n=21), living in the vicinity of TB subjects (n=22) and heavily TB exposed health care workers (n=23)	BCG at birth compared to 2 months of age Adults were not vaccinated	PBMC IFN-γ responses to mycobacterial antigens and controls PBMC IL-13 production (in adults)	PBMC IFN-γ production to PHA and TT (not reported for newborns)	pg/ml	Relevant data/significance not reported	
Vijaya Lakshmi V, *et al* 2005	Case-control study (vaccination was performed retrospectivel y)	Children aged 5-7 years who had been vaccinated with BCG (n=45), unvaccinated (n=31) or had active TB (n=31)	BCG vaccination compared to unvaccinated and active TB patients (vaccination was retrospective)	Lymphoproliferatio n and cytokine responses	IL-2 dependent cell line proliferation supplemented with supernatant from lymphocyte culture stimulated with control antigens IFN-γ levels in supernatant of lymphocyte cultures stimulated with control antigens	Stimulation index pg/ml	Sig higher SI for vaccinated vs non-vaccination normal children , P<0.05 IFN-γ levels sig higer in vaccinated children p<0.01	
Weir *et al* 2004	Randomised, controlled trial, multiple regions	Healthy young adults from Northern Malawi (n= 633, mean age 19 years) and East London and Essex, United Kingdom (n=424, mean age 14 years)	BCG vaccination compared to placebo	Cytokine responses to mycobacterial antigens.	Cytokines (TNF-α, IL-10, IL-1β) from whole blood culture supernatants	Median responses pg/ml	TNF-α- NS IL-10- sig diff from baseline for Malawi subjects for M scrofulaceum and M vaccae only (p=0.015 and 0.02)	Study carried out in the context of the Karonga Prevention Study, a large vaccine trial and epidemiological study of tuberculosis and

								leprosy in the Karonga district.
Weir *et al* Oct. 2008	Single region case-control study	Healthy young adults from East London and Essex, United Kingdom (n=424, mean age 14 years)	BCG vaccination compared to unvaccinated	IFN- γ responses to mycobacterial antigens.	IFN-γ responses to control antigens	%>63pg/ml and %>250pg/ml	NS	
Weir *et al* Jan 2008	Follow-on study of three separate, open label randomized trials	UK adolescents. Unvaccinated 12-14 year olds n=213 Vaccinated 3 years prior, 17-18 year olds n=20 Vaccinated in first year of life, 12-14 year olds n=43 with 212 similar aged children who were unvaccinated as controls	BCG vaccination compared to unvaccinated	IFN-γ from supernatants of whole blood assays stimulated with mycobacterial antigens and controls	Controls used for IFN-γ from supernatants of whole blood assays	pg/ml	NS	

Tetanus toxoid

Author	Methods	Participants	Interventions	Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Armitage *et al* 1993	Case-control, comparing responses in elderly to young adults	Healthy elderly (n= 23, mean age 75) and young adults (n=23, mean age 27)	Tetanus toxoid vaccination depending on prior vaccination history	Immunogenicity, proliferation and blastogenesis	Lymphoproliferation to PHA and ConA	cpm	Sig decrease between groups after vaccination but results pre and post vaccination not reported	
Borut *et al* 1980	Case-control, comparing agammaglobulinaemic subjects to immunised and unimmunised subjects. Retrospective vaccination	Agammaglobulinaemic (n=7, aged 12-27 years), unimmunised (n=9, aged 4-20 weeks), and immunised (n=64, age 8 weeks - 50 years)	Vaccination status determined by history (any individual who received TT within 10 years prior to the study was considered immunised)	Skin testing, proliferation, monocyte chemotaxis and immunogenicity	Monocyte chemotaxis	Number of cells per high powered field	Sig higher monocyte chemotaxis in though with positive Tetanus toxoid skin test, $p<0.01$.	
Chollet *et al* 1979	Case-control	Healthy adults (n=24 in the vaccine arm and n=51 controls)	Boosting with tetanus toxoid compared to no boosting	Lymphocyte proliferation, phenotyping (by electrophoresis), cytotoxicity, and rosettes	B, T1 and T2 cell phenotypes by electrphoresis Lymphoproliferation to PHA, PWM, ConA	% Stimulation index	Significant increase in T2 population over time in 6 of the vaccinated group.	
Chui *et al* 2004	Case-control	Healthy adults cases (n=6, 39.8±7.2 years) and controls (n=6, 36.8±5.4 years)	Flt3-ligand and tetanus toxoid vaccination compared to tetanus toxoid vaccination only	Skin tests, proliferation, IFN-γ and immunogenicity.	Lymphoproliferation to PHA IFN- γ ELISpot to PHA, HBsAg and media alone	Stimulation index Spots per 100 000 cells (not reported)	Not reported	

Cooper *et al* 1998	Case-control study conducted within two Ecuadorian communities	*Onchocera volvulus* infected (n=19, median age 36, range 15-75 years) and uninfected (n=20, median age 36, range 17-67 years) adults	Tetanus toxoid vaccination (2 doses 1 month apart) in both cohorts	Proliferation, cytokines and immunogenicity	Positive and negative controls of PBMC proliferation assays (data not reported in manuscript). PBMC cytokine production	Antigen stimulated assays were reported as geometric mean stimulation index with 95% CIs Median (pg/ml) and 95% CIs	NS NS	
Di Genova *et al* 2006 (Also BCG)	Cohort study	Healthy adults (n=12, age range 31-44 years), all had previously received TT vaccination over 5 years prior. All subjects had also received BCG vaccination	Tetanus toxoid vaccination	PBMC and T cell proliferation and cytokine responses	Memory T cell proliferative responses to *Candida albicans* IFN-γ, IL-2 and IL-13 responses to *C. albicans*, and controls	Mean stimulation index ± SD Number of spots/2x10^5 PBMC	Individual results given, overall significance not reported.	
Fernandez *et al* 1994	Cohort study	Healthy males (n=3, age range 25-40 years)	Tetanus toxoid vaccination in all subjects (1 subject received TT 2 years prior, and 2 subjects received TT 8-9 years prior)	Immunogenicity, proliferation, cytokine production	Lymphoproliferation to PPD PBMC production of TNF-β and IL-2 in the presence of media ± IL-2	CPM Number of positive cells/100 000 PBMCs	Significance not reported	
Fevrier *et al* 1977	Cohort study	Adults (n=7, age range 30-40 years)	Tetanus toxoid vaccination. 2 subjects never previously vaccinated.	Lymphoproliferation (including B and T cell subsets)	Positive and negative control lymphocyte (non-separated, B and T cell) proliferation	Mean cpm ± SD	Significance not reported	
Gentile *et al* 2006	Case-control study	Adults with (n=15, mean age 27 years) and without (n=15, mean age 27 years)	Tetanus toxoid vaccination in all subjects	PBMC cytokine responses, skin testing to allergens and TT	IFN-γ and IL-13 responses to PHA	Mean (pg/ml)	Some between group differences at certain time point. Before and	

		allergic rhinitis		immunogenicity			after results not compared.	
Livingston *et al* 2013	Single centre, cohort study	Healthy adults (n=8 mean age 33 ±10 years)	Tetanus toxoid vaccination.	PBMC proliferation, cytokine responses, cellular phenotyping and *in vitro* antibody production	PBMC proliferation to PBS CD4+CD3+ T cell Proliferation to PBS Intracellular CD4+CD3+ T cell IFN-γ, TNF-α, IL-2, IL-17, IL-10, IL-5, IL-13 and IL-4 produced to PBS Intracellular PBMC IFN-γ, TNF-α, IL-2, IL-17, IL-10, IL-5, IL-13 and IL-4 produced to PBS	Percentage CD25+ and CD69+, CD3+CD4+ T cells Percentage CD3+CD4+ T cells Percentage CD3+CD4+ T cells pg/ml	Significant differences between PBS and TT stimulation only reported.	
Mahalingham *et al* 2010	Randomised, double blinded, controlled trial	Healthy females (n=108, age range 18-25 years)	Tocotrienol-rich fraction supplementation (palm oil) compared to placebo. All subjects received Tetanus toxoid vaccination	Lymphoproliferation, cytokines in culture supernatants, TT immunogenicity and plasma Vitamin E levels.	IFN-γ and IL-4 production in response to ConA IL-6 production in response to LPS	pg/ml pg/ml	Sig increase in IFN-γ over time, p<0.001, and decrease in IL-2, P<0.001 for both groups. Sig difference between groups (all vaccinated) for IL-6 (p<0.05)	Supported by a grant from the Malaysian Palm Oil Board

Measles

Author	Methods	Participants	Interventions	Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Bertley *et al* 2004	Follow on to a randomised, controlled trial. Samples taken before vaccination, at 9 months and at 5 years.	5 year old children in Sudan who had previously taken part in a measles vaccine RCT. Recruited from 6/14 of the communities in the original trial.	At 5 months of age: Connaught high titer measles vaccine, n=61 or meningococcal A+C vaccine as control, n=59. All received standard titer Schwarz vaccine at 9 months. A third group from the original trial (single high dose EZ vaccine) were not included.	Neutralizing antibodies. Lymphoproliferation to measles virus (at 5 yrs only)	Lymphoproliferation to Vero control stimulus.	CPM	NS (comparable across groups, details not given)	Follow-on study
Gans *et al* 1999	Cohort study, all infants vaccinated and blood taken before and 12 weeks after immunisation.	Healthy infants in the USA aged: 6months (N=60), 9 months (N=46) 12 months (N=56) And Healthy adults aged 20-40years who had previously had at least one measles vaccine (N=29).	6 and 9 month-old infants: Monovalent Measles vaccine (Attenuvax) 12month-old infants-:MMR Adults: no vaccination given	Measles specific T cell proliferation, IL-2 and IFN-γ production. Effect of passive measles antibody and rhIL-12 on above. T cell proliferation to PHA (not fully reported). Measles antibody titres.	IL-12, IFN-γ, and T cell proliferation to PHA (not fully reported).	Mean CPM with standard error and pg/ml. .	NS (p value not given)	Post vaccine samples available for 134/162 infants but not all tested for all assays. Non-specific effects not fully reported.
Gans *et al* 2004	Case control	Cases:	Cases:	Measles antibody,	T cell proliferation	Results not	Results not	Not all sample

	study. Cases received measles vaccine at 6 or 9 months then MMR at 12 months. Blood taken before and 12 weeks after first vaccination then 24 weeks after MMR. Controls enrolled at 12 months. Bloods taken before and 24 weeks after MMR.	Infants receiving well-child care in California aged 6 (N=32) or 9 (M=23) months. Controls: 12month infants (N=83)	Monovalent Measles vaccine (Attenuvax) at 6 or 9 months followed by MMR at 12 months Controls: MMR at 12 months	(passive and non) after 1 and 2 doses. T cell proliferation to measles antigen and PHA (not reported).	to PHA (not reported).	reported	reported	sizes allowed for full immunological evaluation. Non-specific effects not reported.
Hennino *et al* 2007	Prospective, double-blinded, randomized, placebo-controlled	10-14month-old males and female infants in the USA with atopic dermatitis, N=12,	Single Schwartz strain measles vaccination at 10-14months or placebo (physiological serum)	Severity of atopic dermatitis, seroconversion, serum levels of CCL18 and E-selectin 1 month post vaccination.	Severity of atopic dermatitis, seroconversion, serum levels of CCL18 and E-selectin 1 month post vaccination	Individual SCORAD eczema severity score for 6 randomly chosen infants. Serum levels of CCL18 and E-selectin in ng/ml for six participants before and after treatment.	NS CCL18- sig decrease in 2 individual measles treated subjects compared to baseline(p=0.0183 and 0.0011)	All children had atopic dermatitis
Hussey *et al* 1996	Cohort study with 3 groups (different vaccine and	Healthy infants attending 2 immunisation clinics in Cape	Group 1: Edmonston – Zagreb vaccine (medium titre) at	Measles antibody responses. Proliferation, IL-2 receptor, CD4, CD8,	Proliferation, IL-2 receptor, CD4, CD8, β2 microglobulin and Neopterin in	Median CPM, U/ml (sIL-2r, CD4, CD8), mg/L (β2 microglobulin) and	Group 2 and 3: sig decrease in PHA proliferation at 3m compared to own	Study commenced before WHO recommendation

	ages). Blood taken before, 2 weeks and 3 months after vaccination.	Town, SA (one in high measles prevalence area and one in low measles area) Group 1: (high prevalence) 6months-old, N=38. Group 2: (high prevalence) 6months old, N=26. Group 3: (low prevalence) 9 months-old, N=24.	6 months Group 2: Schwarz vaccine (medium titre) at 6 and 9 months Group 3: Schwarz vaccine at 9 months.	β2 microglobulin and Neopterin in response to PHA. Lymphocyte subsets. Comparison between males and females.	response to PHA. Lymphocyte subsets.	nmol/L (Neopterin).	group baseline, p=0.013, 0.002 Group 3: sig increases in sol CD8 (p=0.02) and β2 microglobulin (p=0.04) from baseline. Sig difference between groups for sol IL-2receptor (p<0.001), sol CD4 (p=0.015) and absolute CD8 count (p=0.008).	to withdraw EZ vaccine
Jaye *et al* 2014	Case-control study (disease versus vaccianted)	Children and adults with acute measles. Healthy newborns (n=22)	Measles vaccination at 9 months of healthy children recruited at birth	Cytotoxic T cell assays	Controls used for cytotoxic T cell assays	Data not reported		
Liguori *et al* 1998	Cohort study, All children vaccinated and bloods taken before, 5 and 15 days after vaccination.	Healthy children in Italy, males and females, aged 5-9 years with no history of measles, N=20.	Single Measles vaccine (Rimevax) at baseline.	Measles IgG and IgM. Serum TNF–α, IFN-α and IFN-γ.	Serum TNF –α, IFN-α and IFN-γ.	pg/ml for each individual subject.	NS	Funding source not reported
Lisse *et al* 1994	Case-control, follow-on study of 2 previous trials.	All participants from capital of Guinea-Bissau Cases: From trial 1- all children who had received	Follow-on study, no new interventions	Total white cells and lymphocyte subsets (presented for each trial and by sex).	Total white cells and lymphocyte subsets (presented for each trial and by sex).	Median cell counts/% cells with interquartile range.	NS (cases vs control, some difference found when analysing subgroups by sex and vaccine)	330 of the 854 originally recruited provided samples for this follow-on study. Mortality

		medium titre Edmonston-Zagreb (EZ) vaccine at 4months, now aged 5 years. From trial 2- children who had received either medium or high dose EZ vaccine at 4 months, now aged 3-4years. Controls: Children from previous trials who received standard dose Schwarz vaccine at 9months.						data is presented elsewhere in Aaby *et al* 1993
Njie-Jobe *et al* 2012	Randomised trial comparing children vaccinated at 4 and 9 months or just 9 months. Bloods at baseline, 4, 9 or 9.5 (randomized to tests for memory or effector response), 18, 36, 36.5 and 48 months.	Gambian infants, male and female, initially recruited at 4 months of age, N=132	Group 1 (N=64): Measles vaccine at 9 and 36 months Group 2 (n=68): Edmonson-Zagreb measles vaccine at 4,9 and 36 months NB both groups also received routine EPI vaccines including DTP, HepB, Hib, oral polio and at 9 months, yellow fever.	Measles HAI. IFN γ ELIspot effector responses to measles, uninfected Vero cells, measles fusion peptides and PHA. FOXP3 mRNA expression. IFN γ, IL-10, IL-2Rα and MIP-1β levels. CD8 and CD4 cells expressing IFN γ and/or CD69. NB Not all assays performed at all time-points.	ELISpot IFN-γ response to uninfected Vero cells and PHA (data not reported). Plasma IFN γ, IL-10, IL-2Rα and MIP-1β levels.	Median cytokines level in pg/ml with interquartile range.	NS	

Okada *et al* 2001	Case-control comparing age-matched measles patients with 1-2 year-old children immunized with measles vaccine.	Cases: uncomplicated, acute measles patients in Tokyo area, aged 1-2 years. N=147. Controls: healthy children aged 1-2 years who received measles vaccine. N=32.	Controls received measles vaccine, either AIK-C strain or Schwarz strain, further details not reported.	Total lymphocyte count, CD4, CD8 and B cells. Evidence of apoptosis of PBMC (through analysis of DNA fragmentation). NK cells (data not reported). Measles antibody responses.	Total lymphocyte count, CD4, CD8 and B cells. Evidence of apoptosis of PBMC (through analysis of DNA fragmentation). NK cells (data not reported).	Cells per µL, each point representing average of 3-15 samples. Mean % apoptosis and cell surface expression of Fas, FasL or TRAIL-Rs. Mean cytokine levels in pg/ml (ng/ml for Sol-FasL).	NS	NB for purposes of this SR, only the results for the vaccinated cohort have been included
Ovsyannikova *et al* 2003	Cohort study with two groups of different ages. Randomly assigned to undergo single phlebotomy on one day 0-40 days post intervention.	Healthy infants/children in the USA, male and female. Group 1: 12-15month-old, N=15. Group 2: 4-12year-old, N=42	Single Edmonston strain measles vaccination	PHA stimulated cytokine production (IL-2, IL-4, IL-6, IFN-γ and TNF-α), plasma cytokine levels, measles antibody titres.	PHA stimulated cytokine production (IL-2, IL-4, IL-6, IFN-γ and TNF-α), plasma cytokine levels.	Median cytokine concentration in pg/ml (different children at each timepoint) with interquartile range.	NS over time for each group from bseline to 40 days. Some significant changes at earlier timepoints but returned to baseline. (Also some sig differences between groups, both of which were vaccinated but were different ages).	Each subject had single blood draw therefore results at each time point are different children. Day 0 children (N=2 and 7) did not receive vaccine before blood draw.
Pabst *et al* 1999	Case-control comparing children of mothers who either had natural	Group 1: 6 month-old infants whose mothers were assumed to have had measles, N=61. Group 2a: 6 month-	Group 1 and 2a: Single dose of standard titre AIK-C measles vaccine at 26-32 weeks	Reactogenicity. Measles specific antibody. Blast transformation to measles HA antigen. Production	Production of IFN-γ and IL-10 stimulated by PHA. Blast transformation to TT, non-measles	Pg/ml, mean ± standard deviation. Mean CPM ± standard deviation.	NS	Measles vaccine was given immediately after the third dose of DTP-Polio-Hib.

	measles or were vaccinated against measles plus separate cohort vaccinated with different measles vaccine. All children vaccinated, bloods taken before and 8 weeks after vaccination.	old infants whose mother were known to be vaccinated against measles, N=119. Group 2b: 6 month-old infants whose mothers were known to be vaccinated against measles, N=120	Group 2b: Single dose of standard titre Connaught (CLL) measles vaccine at 26-32 weeks	of IFN-γ and IL-10 stimulated by measles antigen, TT, non-measles infected Vero cells, PHA and Candida	infected Vero cells and Candida.			Part funding provided by Merck-Frost, Canada
Samb *et al* 1995	Case-control study.	Rural Senegalese children who had received EZ vaccine at 5 months (n=73) and children who had received placebo at 5 months followed by Schwarz vaccine (n=70)	Rabies vaccine	Immunogenicity to measles, yellow fever and rabies vaccines. Skin tests. Haematological parameters.	Rabies and Yellow fever immune responses. CD3, CD4, and CD8 lymphocyte counts	YF – Log2 neutraliazation antibodies Rabies – Log10 reduction neutraliazation x10^6 cells/L	NS Significant difference: girls vs boys for both EZ and standard	
Schnorr *et al* 2001	Quasi-RCT comparing unmatched controls with those immunised at 4 and 9 months or 9	Bangladeshi infants aged 6 and 9 months of age. NB unmatched controls median age 10.1 months (range 6-18m). Total n= 78	Group V6: Standard titre measles vaccine (2/3 had standard titre EZ and 1/3 had Schwarz) at 4 and 9 months, n=24.	Measles antibody levels. Delayed hypersensitivity skin test. Cytokine responses	Delayed hypersensitivity skin test. Cytokine responses in response to stimulation with PHA.	Geometric mean cytokine production I pg/ml	Vaccines sig more anergy to candida in skin test but not other antigens. NS T-lymphocyte subsets Inconsistent sig	All received vitamin A on recruitment

	months only. Comparison of Blood at recruitment for controls or 7 days after vaccination for vaccinated groups and at 6 and 24 weeks for all groups.		Group V9: As above at 9 months only, n=25 Controls: no vaccination, n=29. All received Vitamin A on recruitment.	in response to stimulation with PHA.			difference at different times points for CD25, 69, 71 and 30. Sig increase IL-10 in vaccinated groups at week 6.	

MMR

Author	Methods	Participants	Interventions	Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Nakayama *et al* 1990	Cohort study. All children vaccinated and blood taken before and 6 weeks after vaccination. A subset of children also had bloods at 3,7,10 days and 2,3,5 and 10 weeks after vaccination.	Healthy children who visited outpatients in Tokyo aged 1-5years, N=229.	MMR vaccine containing measles AIK-C strain, Mumps Hoshino strain and Rubella Takahashi strain, single dose at baseline.	IFN-α responses to measles, mumps and rubella- subset of 11 children only. Measles and Rubella serum antibodies (paired serum for all subjects).	Non-stimulated controls (not reported).	Results not reported.	Not reported	No non-specific effects are reported and only non-stimulated control experiments were performed. Funding source not reported
Pabst *et al* 1997	Cohort study, all infants vaccinated and blood taken before and either 14 (N=32), 22 (N=32), 30 (N=27) or 38 (N=33) days after vaccination.	12month infants from Edmonton, Canada, N=124	MMR given within 4 weeks of first birthday	Blast transformation of PBMC to measles antigen, Vero cell control antigen, TT and Candida antigen. Production of sIL-2r, IFN-γ, IL-4 and IL-10 stimulated by PHA. CD4, CD8 and NK cells.	Blast transformation of PBMC to Vero cell control antigen, TT and Candida antigen. Production of sIL-2r, IFN-γ, IL-4 and IL-10 stimulated by PHA. CD4, CD8 and NK cells.	CPM, mean and standard error of mean. pg/ml, mean and standard error of mean. % of PBMC, mean and standard error of mean.	Significant change over time Significant change over time Significant change over time	Not all children assessed at each time point. Part funding provided by Merck-Frost, Canada
Rager-Zisman *et al* 2003	Cohort study, all children vaccinated and blood taken before	Healthy Israeli children; age 6.14 (±0.35) years with previous MMR vaccination in	Single MMR vaccination (MMRII) Note: subjects	Measles, mumps and rubella IgG, total IgM, IgG and IgE. White cell count, CD8, CD4	White cell count, CD8, CD4 and CD4:CD8.	Median white blood cell count (x1000/μl), % CD4, %CD8 and CD4:CD8 ratio with	Sig decrease in total leukocytes ($p<0.0001$), %CD4 ($p=0.028$) and %CD8 cells	Number of children tested varies by assay (N=28-38)

	and 30 days after vaccination.	infancy. N=38	had previously received MMR in infancy and had recently had a tetanus booster	and CD4:CD8. Lymphoproliferative responses to PHA and TT. NK cells and NK specific activity.	Lymphoproliferative responses to PHA and TT. NK cells and NK specific activity.	interquartile range before and after vaccination. Mean and standard error of the mean CPM. Mean and standard error of mean %CD56 cells.	(p=0.041) before and after immunisation. Response to TT sig increase (p=0.006) Sig increase in CD56+ cells (p=0.01)	

DTP and DT

Author	Methods	Participants	Interventions	Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Dirix *et al* 2009	Cohort study. Blood taken at 2 (before vaccination, N=42), 3 (n=28) and/or 6 (N=24) months of age and at around 13 months (N=5)	Infants in Belgium aged 2-13 months with HIV positive mothers but who were HIV negative themselves, N=63.	Infants had previously received Tetravac (tetanus, acellular pertussis, diphtheria, polio vaccine) and H influenza type B polysaccharide vaccine at 2, 3 and 4months and recombinant Hepatitis B vaccine at 3 and 4 months.	PHA, FHA and PT induced IFNγ and IL-12p70. Spontaneous IL-10 secretion. Effect on IFNγ secretion in presence and absence of anti-IL-10 antibody. IL-10 polymorphisms.	PHA induced IFNγ and IL-12p70. Spontaneous IL-10 secretion.	Median pg/ml with interquartile range. NB PHA data reported based on spontaneous IL-10 secreting status. IL-10 data reported for individual infants.	NS/not fully reported	All children had been involved in previous studies on cellular immune responses to pertussis vaccine All had received 6 weeks of Zidovudine prophylaxis until HIV status was confirmed. Part funded by Sanofi Pasteur.
Fernandes *et al* 2010	Cohort study. Blood taken 7 days before, 7 and 28 days after vaccination.	Healthy males aged 18-55 years in Canada, N=20.	Single dose of tetanus/diphtheria vaccine.	Frequency of B lymphocyte subsets. IgG, IgA and tetanus/diphtheria specific antibody secretion. IgA and IgG antibody to polio virus and HSV.	Frequency of B lymphocyte subsets. IgA and IgG antibody to polio virus and HSV.	Mean absolute number or % cells ±standard error of mean. Spots/1000 PPC for 3 subjects, error bars are standard error of mean.	NS over 28 days Sig increase at day 7 but not at day 28	Adult only study. Funding source not reported.
Fryauff *et al* 1998	Sub study of anti-malarial RCT. Subjects in anti-malarial trial	Healthy Adult male transmigrate farmers of Javanese/Sundanese ethnicity, who	Non-control subjects had previously randomised to weekly	Lymphocyte proliferation in response to PHA, TT, M. TB PPD and TT subunit peptides	Lymphocyte proliferation in response to PHAand M. TB PPD	PHA and PPD data not reported.	Not reported	Adult only study. Sub study of anti-malarial trial.

	were immunised while controls (not taking part in malaria trial) were not. Blood taken before, 1 and 7 months after immunisation.	were taking part in a malaria prophylaxis trial, N=72 and control subjects not having malaria prophylaxis, N=20.	chloroquine, daily primaquine or placebo for 12 months. These subjects received Tetanus and diphtheria toxoid vaccine 11months after starting the assigned drug regimen. Control subjects did not receive any intervention.	(P2, P30 and P2P30)				
Halasa *et al* 2008	Prospective, randomised, pilot study investigating an additional dose of DTP at birth. Safety data collected after every dose and blood samples at birth, 6,7,17 and 18 months.	Infants aged 2-14 days, n=50.	Group 1: DTaP and hep B at birth Group 2: Hep B only at birth. NB Both groups received routine vaccines including DTaP at 2,4,6 and 17 months.	Adverse reactions. IgG to PT, PHA PRN and Fimbriae 2/3. PRP Hib capsule antigen. Pneumococcal capsule antigens, diphtheria and tetantus toxoids. Neutralization assays for polio and hep B surface antibodies (7 months only).	Polio neutralisation assays, pneumococcal serotypes 6, 14, 23 and Hep B antibodies at 7 and 18 month visits.	Geometric mean concentrations	Higher GMC for pneumococcal serotype 14 in controls at 7 months (p=0.035) and higher microneutralisation titres in controls for poliovirus 1 and 3 at 18 months (p=0.39 and 0.041)	At 7 months , 2 subjects in the experimental group and 1 control had an additional Hib dose as protective antibody titers not achieved.
He *et al* 1998	Case-control study comparing children and adults either vaccinated	Disease cases (total N=8): 13 year old children (N=6) and adults (n =2, aged 26 and 60 years) with culture	Single dose of combined diphtheria-tetanus-trivalent acellular pertussis (DTaP) vaccine	Proliferation in response to pertussis antigen (PT, FHA and PRN), PHA and pokeweed mitogen (latter two	Proliferation in response to PHA and pokeweed mitogen (not reported).	Data not shown for PHA and PWM. Medium only reported for individuals as	NS (p value not given)	All adult vaccinated controls and cases came from authors department.

	against pertussis or with natural pertussis infection and children/adults who did not receive pertussis vaccine. Blood taken before and 1 month after immunisation	confirmed pertussis infection. Vaccinated cases (total N=20): Male and female children in Finland, aged 10-12years, N=17. Adult males, N=3. Controls (total N=25) : 10-12year old children who had received DT booster in last month (N=9), Adults (N=8), healthy 13 year-olds (N=6) and newborns (N=2)	given to vaccinated cases	not reported). Cytokine mRNA expression in PBMCs for IFNγ, IL-2, IL-4 and IL-5. IgG antibodies to PT, PHA and PRN.	Cytokine mRNA responses to PHA and unstimulated were not reported	$[H^3]$thymidine incorporation		
Heine *et al* 2011	Randomized, double-blinded, placebo controlled trial comparing oral vitamin D to placebo. All subjects immunised after 9 weeks of supplementation and blood	Adults aged 26-34.5 years	2000IU of oral vitamin D3 oil per day or equal volume of neutral oil. All subjects received tetanus/diphtheria toxoid vaccine after 9 weeks of supplementation.	25-hydroxyvitamin D. TT specific IgG and IgA. Peripheral B and T lymphocytes. T cell activation to stimulation by Staph enterotoxin. TT specific cytokine profile (IL-2, IL-4, IL-5, IL-10, TNF-α and IFNγ). Adverse events. Leukocyte counts	T cell activation to stimulation by Staph enterotoxin and no antigen. Pre vaccination data not reported. Leukocyte counts and IgG, IgA and IgM levels (supp. data)	Medians in pg/ml with interquartile range concentrations	Not reported All NS except monocyte count in placebo group.	Randomized intervention is Vitamin D supplementation therefore no unimmunised controls.

	taken before and 7 days after immunisation.			and IgG, IgA and IgM levels (supp. data)				
Jorgensen *et al* 2013	Sub-study within a larger randomised double blind controlled trial	Infants six weeks of age (n=480), either prior or after DTP vaccination	Randomised to Vitamin A supplementation or placebo	Cytokine production. Leukocyte counts.	TNF-α, IL-10, Il-5, IL-13 and IFN-γ responses in culture Lymphocytes, granulocytes, Eosinophils and monocytes	Geometric mean (pg/ml) Mean and SD	NS NS	
Lin *et al* 1997	Randomized, controlled trial with three groups, including control. 15/80 subjects bled before and 1 month after vaccination.	Healthy medical personnel in Taiwan, aged 20-40 years, N=80.	Single dose of: Group 1: Td + full strength acellular pertussis vaccine Group 2: Td+half strength acellular pertussis vaccine Group 3: Td alone as control	Adverse reactions. Anti-PT and anti-FHA antibodies. Lymphocyte proliferation to PT, FHA, Con-A, PHA and PWM. Cytokine production (IFN γ, IL-4, IL-5) to Con-A, PT and FHA.	Lymphocyte proliferation to Con-A, PHA and PWM. Cytokine production (IFN γ, IL-4, IL-5) to Con-A.	Stimulation index CPM, mean ±standard error. pg/ml, mean ±standard error.	NS	Adult only study. Designed to examine different pertussis vaccines but all received Td including controls.
Rowe *et al* 2000	Cohort study, all vaccinated, blood samples at 2, 3, 6 and 12 months.	Healthy infants in Australia, n=55	DTaP at 2, 4 and 6 months. Infants also received oral polio and Hib titre vaccines.	IL-4, IL-5, IL-6, IL-9, IL-10, IL13 and IFN γ stimulated by TT and PHA. IL-4 and IL-9 mRNA.	IL-4, IL-5, IL-6, IL-9, IL-10, IL13 and IFN γ stimulated by PHA.	Median pg/ml (test minus control cultures) with 10^{th}, 25^{th}, 75^{th} and 90^{th} percentiles. for positive responders	Sig increase at 12 months compared to earlier time points for IL-5 and IL-13, p=0.01 and 0.001	
Yousfi *et al* 2005	Case-control study comparing responses of elderly and young adults to mild	Cases: Elderly, male and female volunteers (mean age 70±4), N=7. Controls: Young, male and female volunteers (mean	Single dose DT-Polio and Typhim Vi vaccine.	Acute phase proteins (CRP, AGP, Fibrinogen, α1-Antitrypsin, Haptoglobulin, Albumin, Transthyretin,	Acute phase proteins (CRP, AGP, Fibrinogen, α1-Antitrypsin, Haptoglobulin, Albumin, Transthyretin,	Acute phase protein in mg or g/L, means ±standard error of mean.	Sig increase in CRP (p=0.003), AGP (p=0.0007), fibrinogen (p=0.004), haptoglobin (p=0.0023) and	Adult only study. Designed to compare elderly with young adults. Vaccine used

	inflammatory stress (i.e. vaccine). Blood taken 7 days before and 2 days after vaccination.	age 23 ± 2).		Transferrin). White blood cells counts. Plasma cytokine levels (TNFα, IL-6, IL-10). IL-6 and IL-10 production by LPS stimulated whole blood. IFN-γ production by PHA stimulated whole blood.	Transferrin). White blood cells counts. Plasma cytokine levels (TNFα, IL-6, IL-10). IL-6 and IL-10 production by LPS stimulated whole blood. IFN-γ production by PHA stimulated whole blood.	Cells/L, means ±standard error Cytokine levels ng/L or pg/ml, individual values with mean.	Transthyretin (p=0.01). Sig increase in monocytes (p=0.007), lymphocytes (p-0.002) and neutrophils (p=0.04) Plasma TNFα- NS. IL-6- sig increase (plasma level and LPS stim), p=0.008. IL-10-sig increase (plasma levels and LPS stim), p=0.05. IFN γ- sig increased (p=0.005).	included Typhoid. Funding source not reported.
Zorzeto *et al* 2009	Prospective, randomized, double-blinded, phase I comparative trial. Blood sample taken at 7 months of age (1 month after last vaccine).	Infants in Brazil, male and female, aged 2.1(±0.5 and 0.3)months N=247	Immunisation at 2, 4 and 6 months with either conventional whole cell pertussis vaccine or pertussis vaccine with low LPS content. Both vaccines also contained Diphtheria and tetanus toxoids.	T cell, CD3+ CD4+, CD8+ and γδ+ cell proliferation when stimulated by pertussis and PHA. Adverse events. IFNγ, TNF-α and IL-10 concentrations in response to pertussis and PHA. Anti-PT IgG titres. % protected against Diphtheria	T cell, CD3+ CD4+, CD8+ and γδ+ cell proliferation stimulated by PHA. IFNγ, TNF-α and IL-10 concentrations in response to PHA.	Median % of specific T cells with 95% CI. Medians of CD3+ blasts with individual points also plotted. Median pg/ml with individual points also plotted.	NS	

				and tetanus.				

Pertussis

Author	Methods	Participants	Interventions	All Outcomes	Non-specific outcomes	Method of reporting non-specific outcomes	Difference in NSE outcome	Notes
Di Tommaso *et al* 1997	Follow-on of two phase 1 trials. Subjects bled either 6, 12, 18 and 24 months (monocomponent vaccine group) or 1, 2.5, 12, 18 and 54 months (tricomponent group) after second vaccine.	Healthy adults, male and female, 25-38 years, who previously participated in one of two studies, N=8.	Subjects had previously received two doses, six weeks apart, of either a monocomponent pertussis vaccine (N=4) or tricomponent pertussis vaccine (N=4).	PT neutralizing antibodies. IgG antibodies to PT, FHA and 69K antigens. Proliferation of PBMC in response to PT and TT, FHA and 69K.	PBMC proliferation response to TT	Stimulation index for each participant at each time point for those who had previously received monocomponent vaccine only.	Significance not reported	Authored by IRIS, the Chiron-Vaccines Immunobiology research Institute

Appendix C: Methodological appraisal of included studies

BCG

Author		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
Akkoc *et al* 2010	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Full term healthy newborns from a single centre	No information on randomisation methods	Open trial	No information on management of missing data	No information on adjusting for multiple analyses	
Anderson *et al* 2013	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
	Support for judgement	Subgroup of a larger trial with proportions of children taking part in subgroup trial varying by month on inclusion in the larger trial.	Randomized but no details given. Even with randomization, BCG group had significantly less hospitalisation and more often received micronutrients	Not blinded	Different children sampled at different timepoints	Multiple subgroup analyses	Part of the REVAC trial. During the study there was a national campaign to supplement with vitamin A and give missing vaccinations. Iron was also distributed to a subgroup in connection with a malaria trial.
Black *et al* 2001	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Unclear risk	High risk	Unclear risk
	Support for judgement	Database was screened for eligible individuals	No details of randomisation method	No details of blinding process. Assume that participants were at	84% follow-up rate at 1 year, however variable numbers reported for each assay	Different numbers of subjects reported for each assay without explanation	Overall limited amount of information.

				least blinded to intervention			
Black *et al* 2002	**Authors judgement**	Low risk	Low risk	Low risk	Low risk	Unclear risk	
	Support for judgement	Database was screened for eligible individuals in Malawi. In the UK recruitment was via a school vaccination program.	Randomised in blocks of six	Staff and subjects blinded to intervention	Probably a complete set of data	Those subjects who had positive skin tests were recruited in to the control groups	
Burl *et al* 2010	**Authors judgement**	Low risk	Low risk	Unclear risk	Low risk	Low risk	
	Support for judgement	Full term healthy newborns from a single centre	Randomised in blocks of 20	Open trial	87 out of 103 completed 9 month time-point	Corrected for multiple analyses	
Burl *et al* (Aug.)	**Authors judgement**	Low risk	Low risk	Unclear risk	Low risk	Unclear risk	
	Support for judgement	Recruited at delivery from a single hospital.	Randomised in blocks of 20	Open trial	85/103 were followed-up at the final 20-28 month time-point	Statistical corrections made for multiple analyses Possibly more subjects excluded in group 2 due to tuberculosis exposure. i.e at 4.5 months Grp 1 n=51/53 Grp 2 n=39/50	
Djuardi *et al* 2010	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear	
	Support for judgement	Non-random. Part of a cohort to examine immune responses to helminths in children	Non-random	Open trial	98 out of 147 children were followed up at the 2 tear time-point	No information	
Elliot *et al* 2011 (BCG and TT)	**Authors judgement**	High risk	High risk	Unclear risk	High risk	Low risk	High risk
	Support for judgement	Random sequence generation was for mother's treatment. Infants were not randomized to any intervention	Non-random	Open trial	1506 out of 2345 completed the BCG arm 1433 out of 2345 completed the TT arm	Missing data described.	Mothers received various numbers of vaccinations during pregnancy. There is no data

							on whether this may affect the infants subsequent response to vaccine
Faustman *et al* 2012	**Authors judgement**	Low risk	Low risk	Low risk	Low risk	Unclear risk	
	Support for judgement	Concealed allocation	Randomisation scheme prepared by the hospital pharmacy	Staff and subjects blinded to intervention	All subjects accounted for	A placebo treated subject developed primary EBV and was reported exclusively	
Fjallbrant *et al* 2007	**Authors judgement**	Unclear risk	High risk	High risk	Low risk	Unclear risk	
	Support for judgement	Subjects selected from 123 TST negative healthcare students based on matching previously vaccinated and not groups.	Non-random	Non-blinded	No apparent attrition.	Non-specific data not reported	
Gruber *et al* 2000	**Authors judgement**	Unclear risk	High risk	Unclear risk	High risk	Low risk	
	Support for judgement	Prospective cohort but 38% selective as high risk for atopic disease by family history	Vaccinated children had received BCG as they were at increased risk of TB and were more likely to be non-German	Unclear if blinded.	774/1314 included in this analysis as completed all scheduled examinations. Significant difference noted between these children and cohort as a whole in breastfeeding and vaccination rates.	Addresses aims, corrects for multiple analyses.	
Hoft *et al* 1998	**Authors judgement**	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	
	Support for judgement	No information on recruitment	No information on randomisation method	Double blinded	53/54 participants completed the study. Some missing data not accounted for	Sub classified subjects in to responders and non-responders	
Hoft *et al* 1999	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	

	Support for judgement	No information on recruitment	No information on randomisation method	One arm was open label. Three arms were double blind	65/66 participants completed the study. Some missing data not accounted for	Most of the ELISA data is not reported.	
Hussey *et al* 2002	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Doesn't state specifically how subjects were identified	Non-random	Open trial	Data missing for some assays.	Didn't correct for multiple analyses.	
Kagina *et al* 2009)	**Authors judgement**	Low risk	Low risk	Unclear risk	Low risk	Unclear risk	
	Support for judgement	Approached mothers at antenatal clinic	Randomly allocated	Open trial	Relatively acceptable exclusions at each time-point documented	No information on a priori analysis	
Kleinnijenhuis *et al* 2012	**Authors judgement**	High risk	High risk	Unclear risk	Low risk	Low risk	
	Support for judgement	Non-random	Non-random	Open trial	Small group size with no apparent attrition	No information on adjustment for multiple analyses	Small group size and no information on group subject composition
Lalor *et al* 2009	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Recruitment in the UK through health centres. In Malawi recruitment took place at a single hospital	Non-random	Open-trial	No information on missing data	Sub-set of Malawi cohort who were vaccinated at the same time as the UK cohort were compared	Different age of vaccination in UK compared to Malawi
Lalor *et al* 2010	**Authors judgement**	High risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk
	Support for judgement	Subgroups of a larger study	Non-random	Not blinded	Single time-point an d all results appear present	Unstimulated control data not reported	Infants had previously taken part in an IFN gamma study
Lalor *et al* 2011	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Low risk	High risk
	Support for judgement	Subgroup of a larger study where recruitment was in the UK through health centres. In Malawi recruitment took place at a single hospital	Non-random	Open-trial	No information on missing data	No information	Different age of vaccination in UK compared to Malawi

Libraty *et al* 2014	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	
	Support for judgement	Selected form a nested study due to specific attributes	Not random	Not blinded	No information on management of missing data	All data appear to be reported	
Lowry *et al* 1998	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk	Unclear risk
	Support for judgement	Doesn't state specifically how subjects were identified	Randomized but no details given	Unclear if blinded	Blood from 62/76 subjects was tested for lymphoproliferation but 76/76 for serological studies	Non specific data not reported	15 subjects were revaccinated at 16 weeks
Marchant *et al* 1999	**Authors judgement**	Low risk	Low risk	Unclear risk	High risk	Unclear risk	
	Support for judgement	Healthy newborns from a single centre	Randomised in blocks of 6	Open trial	48 out of 137 were studied at the 1 year time-point	Adjusted for multiple analyses, but no information on a priori analysis and ITT analysis	
Marks *et al* 2003	**Authors judgement**	High risk	High risk	High risk	Unclear risk	Low risk	
	Support for judgement	Mothers of potential participants identified by hospital records	Non-random	Retrospective open trial. No information regarding masking of laboratory staff.	Attempted to gather information from those who didn't respond to the survey.	A small proportion of the cytokine data was not included in analysis due to low responses in controls. Corrected for a wide variety of confounders	
Miles *et al* 2008	**Authors judgement**	High risk	Unclear risk	Unclear risk	High risk	High risk	High risk
	Support for judgement	All samples from infants recruited into a larger CMV study	Non-random	Not blinded	133 infants had blood taken at some point but largest number of samples at any one time-point was 87.	NHDF results not reported	All infants were infected with CMV
Miles *et al* 2009	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Non-random	Non-random, no unvaccinated controls	Open trial	No information on management of missing data	Infants that were diagnosed with HIV were excluded. ELISpot data classified into high and low responders	HIV positive mothers and children born to them all received anti-retroviral

							therapy
Ota *et al* 2002	**Authors judgement**	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Healthy newborns from a single centre	Randomised in blocks of 6	Open trial	85 out of 151 were followed-up at 4.5 month time-point	Selective data for responses to HBV reported	
Smith *et al* 2012	**Authors judgement**	High risk	Unclear risk	Unclear risk	Low risk	Low risk	
	Support for judgement	Doesn't state specifically how subjects were identified	Non-random	Open trial	Missing data accounted for.	Adjusted for multiple comparisons	
Soares *et al* 2013	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	
	Support for judgement	Recruitment antenatally, of mothers attending maternity units	Not random	Not blinded	11/90 lost to follow-up and 6/90 exclusions	Data appear to be complete	
Steenhuis *et al* 2007	**Authors judgement**	High risk	Unclear risk	Low risk	Low risk	Unclear risk	High risk
	Support for judgement	Newborns were recruited antenatal from parents with allergic disease	Randomly allocated although no information on randomisation method	Parents not blinded but principle researcher was.	115/121 completed the study	Only 58 tested for eosinophils at 18 months	Unable to recruit required 200 participants therefore explorative analysis only. 19 were revaccinated at 4 months.
Tastan *et al* 2005	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
	Support for judgement	Neonates were randomly chosen but not further details of selection process	Randomised but no details given	Not blinded	36/40 completed study	All results appear present	Funding source not reported
van den Biggelaar *et al* 2009	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Very few details on participant selection. Significant differences at baseline between PNG	Not random	Not blinded	Some participants missing in outcome reporting with no explanation	Outcomes not clearly defined	

		and Australian newborn cohorts for caesarean delivery and head circumference					
Vargas *et al* 2004	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	
	Support for judgement	Children recruited from several schools and diagnosis confirmed with pre-specified criteria.	Unclear if properly randomised (alternate allocation to group)	Unclear if blinded	67/82 completed study. Reasons for withdrawal given.	All results appear present	
Vekemans *et al* 2004	**Authors judgement**	Unclear risk	Low risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	No information on selection procedure	Randomly allocated although no information on randomisation method	Open trial	No information on management of missing data	No information	
Vijaya Lakshmi V, *et al* 2005	**Authors judgement**	High risk	High risk	High risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Non-random	Non-random. Subjects possibly selected based on prior vaccination history	Open trial (retrospective)	No information on management of missing data	Subjects classified into responders and non-responders	Selection bias due to prior vaccine receipt
Weir *et al* 2004	**Authors judgement**	Low risk	Low risk	Low risk	Low risk	Unclear risk	
	Support for judgement	Database was screened for eligible individuals in Malawi. In the UK recruitment was via a school vaccination program.	Randomised in blocks of six	Staff and subjects blinded to intervention	Probably a complete set of data	Those subjects who had positive skin tests were recruited in to the control groups	
Weir *et al* 2008	**Authors judgement**	Low risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Recruitment was via a school vaccination program	Allocation dependent on response to skin testing	Open trial	No information on missing data	No information on adjustment for multiple analyses	Bias towards subjects with a Heaf grade of 2 or above being included into

							the control group.
Weir *et al* 2008	**Authors judgement**	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
	Support for judgement	School vaccination programs	Random allocation	Open label	Only subjects with data at each time-point were included	Not all antigens and controls reported for all cohorts	Study reports responses at different time-point by following up cohorts from three separate studies

Tetanus Toxoid

Author		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
Armitage *et al* 1993	**Authors judgement**	High risk	High risk	High risk	Unclear risk	Unclear risk	
	Support for judgement	Non-random	Non-random, different number of vaccination doses received depending on prior history	Open trial, staff participating in trial	No information	No information	
Borut *et al* 1980	**Authors judgement**	High risk	High risk	High risk	Unclear risk	Unclear risk	
	Support for judgement	Non-random	Non-random. Differing age ranges. Vaccination status determined on history	Open trial, staff participating in trial	No information	No information	
Chollet *et al* 1979	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Non-random	Non-random	Open trial	No information	No information	General lack of information on subject demographics and study design
Chui *et al* 2004	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Non-random	Non-random	Open trial	Missing information for 2 participants	No information	Small cohort sizes
Cooper *et al* 1998	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Non-random	Non-random. Groups defined on presence of	Open trial	No information	No information	

			Onchocera volvulus infection				
Di Genova *et al* 2006 (Also BCG)	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	High risk	
	Support for judgement	Non-random	Non-random, cohort	Open trial	No information	Results selectively reported for maximal response time-point	
Fernandez *et al* 1994	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Non-random	Non-random	Open trial	No information, although missing data unlikely given only 3 subjects in study	No information	Only 3 subjects
Fevrier *et al* 1977	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	High risk	High risk
	Support for judgement	Non-random	Non-random, cohort with very little demographic details reported	Open trial	No information	Selective reporting/conduction of experiments in specific subjects	Small cohort with experiments performed on selected individuals
Gentile *et al* 2006	**Authors judgement**	High risk	High risk	Unclear risk	Low risk	Unclear risk	Unclear risk
	Support for judgement	Non-random	Non-random	Open trial	All subjects completed the trial	Allergy skin testing not reported. Primary outcome defined	Exclusion of those who were on treatment for allergic rhinitis
Livingston *et al* 2013	**Authors judgement**	High risk	High risk	Unclear risk	Low risk	Unclear risk	
	Support for judgement	Non-random	Non-random	Open trial	All participants completed all visits	No information	
Mahalingham *et al* 2010	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Only females selected	No information on randomisation procedure	Double blinded to receipt of palm oil	No information	No information	

Measles

Author		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
Bertley *et al* 2004	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	High risk	High risk	
	Support for judgement	Subjects were followed up from 6/14 of the original communities, chosen at random.	Details of previous randomisation not reported.	Not blinded	Only 37.8% of the original children were followed up at 5 years.	Results from the group who previously received single high dose EZ vaccine are not reported	
Gans *et al* 1999	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Minimal details about subjects (ethinicity etc) and recruitment methods	Not randomized, adult control not matched	Not blinded	Post vaccine samples available for 134/162 infants but not all tested for all assays.	Non-specific effects not fully reported.	Unclear if previously vaccinated adults are used as controls. If so they are not suitable given age, unclear vaccination status etc.
Gans *et al* 2004	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk	
	Support for judgement	Minimal details about subjects (ethinicity etc).	Not randomized. Insufficient information to compare cases and controls.	Not blinded	Not all sample sizes allowed for full immunological evaluation but N is given for each assay.	PHA stimulated results not reported	
Hennino *et al* 2007	**Authors judgement**	Low risk	Low risk	Low risk	High risk	Unclear risk	High risk
	Support for judgement	All children had atopic dermatitis (AD) based on	Randomization file generated by	Double-blinded	5-6 infants out of 12 were tested for	Only 2 placebo subjects had CCL18	One infant seroconverted after

		criteria of Hanifin and Rajka	computer		CCL18/E-selectin due to lack of serum and only 6 were followed up for 6 months for severity of AD scoring	and E-selectin levels reported compared to 4 and 3 measles vaccine receipients	placebo
Hussey *et al* 1996	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Children recruited from two clinics in high and low measles prevalence areas but all children from low risk area received same vaccine and were all 9 months. No details of ethinicity.	Not randomized. Allocatted based on order of attendance. Children form low prevelance area all had same vaccine.	Not blinded	13/88 lost to follow up (2 of which excluded due to serological evidence of measles before vaccination)	Not always obvious from figures how many children have provided results.	All children aged 9 months were from a low prevalence area. Comparisons are made between groups which may not comparable.
Jaye *et al* 2014	**Authors judgement**	High risk	Unclear risk	Unclear risk	High risk	Unclear risk	
	Support for judgement	Very few demographic details	Not random	Not blinded	Initial number of recruits not stated	Not enough information	
Liguori *et al* 1998	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	High risk	Low risk	High risk
	Support for judgement	Insufficient details about recruitment and participants including previous vaccination history.	Not randomized	Not randomized	5/20 lost to follow-up- no details given	Individual results reported for each subject	Funding source not reported
Lisse *et al* 1994	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	High risk	Low risk	
	Support for judgement	Only those taking part in previous study were eligable. Only 330/854 from original study took part. Data for those not taking part not available except for those who died.	Not randomised	Not clear if those analysing samples were blinded to original group.	330/854 from original studies took part.	All results appear present.	Mortality data from this group is presented in Aaby 1993
Njie-Jobe *et al* 2012	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
	Support for judgement	Subjects were recruited from a larger CMV	Randomised but details not given	Not blinded	91/132 completed study. Similar attrition	PHA results not reported.	Despite randomisation,

		observational study.			rates across groups		groups had significantly different median measles antibody titres at baseline
Okada *et al* 2001	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk	High risk
	Support for judgement	Insufficient demographic details (E.g ethinicity and socioeconomic status) to compare groups	Insufficient detail regarding vaccination of controls (eg when vaccinated and how many with each strain)	Not blinded	Each point on the figure represents average of 3-15 patients but unclear if numbers consistent	Control bloods were reportedly taken from 40 age-matched healthy children but no results are given.	NB for purposes of this SR, only the results for the vaccinated cohort have been included
Ovsyannikova *et al* 2003	**Authors judgement**	Low risk	Unclear	Unclear risk	High risk	High risk	High risk
	Support for judgement	Subjects were randomly assigned to timing of blood draw	Not randomized, no control	Not blinded	Each subject had single blood draw therefore results at each time point are different children. Small numbers at each timepoint. Day 0 children (N=2 and 7) did not receive vaccine before blood draw.	As previous	One infant had positive measles antibody titres pre vaccination.
Pabst *et al* 1999	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	High risk
	Support for judgement	300 subjects enrolled over a 20 month period	Subjects in group 2 were apparently randomized to one of two vaccines but all those in group 1 received same vaccine, therefore not all groups comparable	Not clear if blinded	Samples not available for 2/300 at baseline and for 3/300 at 8 weeks. Not all assays performed on every sample- numbers given in paper.	All results appear present	Measles vaccine was given immediately after the third dose of DTP-Polio-Hib therefore any non-specific effects cannot be attributed to measles vaccine alone.

							Group 1 mothers were assumed to have had measles but this was not confirmed (e.g. through testing mothers for antibody).
Samb *et al* 1995	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	
	Support for judgement	Very few details on participant demographics	Randomly selected but no details of methods	Not blinded	Very few details on participants that were not included or missing data	all results appear present, however no evidence of an a priori analysis	
Schnorr *et al* 2001	**Authors judgement**	Unclear risk	High risk	Unclear risk	Unclear risk	High risk	
	Support for judgement	Recruited from larger measles trial	Not randomised. Controls more likely to have acute malnutrition and significantly less likely to have had 3 doses of DTP.	No information on blinding	Higher loss to follow up in control group	Non-significant data not shown	

MMR

Nakayama *et al* 1990	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk
	Support for judgement	Minimal details about subjects (ethnicity, mean age etc.)	Not randomized	Not blinded	Paired sera obtained for all subjects (NB only 11 took part in IFN subset)	Non-specific effects not reported.	Only 11/229 children had blood taken for IFN assays.
Pabst *et al* 1997	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	
	Support for judgement	Minimal details about subjects (ethnicity, mean age etc.)	Not randomized	Not blinded	Not all children assay at each time point. Not clear if all children provided two samples.	All results appear present	
Rager-Zisman *et al* 2003	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Healthy Israeli children but limited information about subjects (ethnicity etc) and 13 had received 2 doses of MMR previously	Not randomized, no control	Not blinded	Number of children tested varies by assay (N=28-38). Due is some cases to lack of serum but details not given.	As previous.	13 children had received two doses of MMR previously due to an epidemic.

DTP and DT

Author		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
Dirix *et al* 2009	**Authors judgement**	High risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Unclear risk
	Support for judgement	Subjects were selected by having HIV positive mothers but being HIV negative. All had received Zidovudine for 6 weeks until HIV status confirmed. All had been included in previous studies.	Not randomised	Not blinded	Not all infants were bled at all time points and not all assays could be performed due to limited blood volumes.	Some assays only report results from 11 subjects.	All children had been involved in previous studies on cellular immune responses to pertussis vaccine. All had received 6 weeks of Zidovudine prophylaxis until HIV status was confirmed. Part funded by Sanofi Pasteur.
Fernandes *et al* 2010	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk	Unclear risk
	Support for judgement	Male only subjects.	Not randomised, no unvaccinated controls.	Not blinded	All completed protocol	Frequency of B lymphocyte subjects is from one representative subject.	Funding source not reported.
Fryauff *et al* 1998	**Authors judgement**	High risk	High risk	Unclear risk	Unclear risk	Unclear risk	High risk
	Support for judgement	Subjects were recruited from a pre-existing malaria prophylaxis trial.	Not randomised, controls differed as not receiving anti-	Not blinded	Attrition rate not reported. Some assays not done (table 1)	PHA and PPD data not shown.	Subset of other (anti-malarial) trial. Unclear if

		All male.	malarials.				control group was part of this trial or not.
Halasa *et al* 2008	**Authors judgement**	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
	Support for judgement	270 parents approached to get sample size of 50.	Randomised but no details given.	Parents blinded, but unclear if study staff were.	42/50 completed study, reasons for withdrawal given.	All results appear present	Part funded by Sanofi- Pasteur
He *et al* 1998	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	High risk	High risk
	Support for judgement	Children randomly selected. Adult vaccinees and controls were convenience sample from department.	Not randomised. Natural infection cases had been symptomatic for a wide range of time.	Not blinded	PHA and pokeweed responses not shown.	Results reported for certain patients only which varies by assay	Controls not well matched for group as a whole.
Heine *et al* 2011	**Authors judgement**	High risk	Unclear risk	Low risk	Unclear risk	High risk	High risk
	Support for judgement	Recruitment from department of Dermatology and Allergy but no details of any medical conditions reported.	Random assignment to Vit D treatment group but no details of process given	Double-blinded (NB blinded intervention is Vitamin D supplementation)	Attrition rates not reported	Subjects with evidence of spontaneous cytokine release were excluded due to assumed subclinical concomitant infection.	Randomized intervention is Vitamin D supplementation therefore no unimmunised controls.
Jorgensen *et al* 2013	**Authors judgement**	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
	Support for judgement	Subjects recruited across six districts. No differences in demographics. Rules for inclusion modified mid-study	Randomised using a code held by the pharmacist.	Double blinded	No information on how many/or if all of those recruited into the main study were approached for this sub-study	Recruitment protocol modified significantly with respect to DTP outcome reported	DTP vaccinated cohort much smaller than unvaccinated cohort
Lin *et al* 1997	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	High risk	Unclear risk	
	Support for judgement	All medical personnel from one Children's hospital in Taiwan.	No details of randomisation process given	Unclear if blinded	Only 15/80 were bled before and after vaccination. However all 80 provide adverse reaction data.	Unclear how many subjects are included in each table.	
Rowe *et al* 2000	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Low risk	High risk	Unclear risk
	Support for	Recruitment details not	Not randomized	Not blinded	Samples were obtained	Results are reported for	Funding source

	judgement	given			from ≥78% of the group at eah time point	positive responders only	not reported
Yousfi *et al* 2005	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk
	Support for judgement	Recruitment details not given.	Not randomised, no unvaccinated controls	Not blinded	Attrition rates not reported however some subjects appear missing.	All results appear to be reported.	Funding source not reported
Zorzeto *et al* 2009	**Authors judgement**	Low risk	Unclear risk	Low risk	Unclear risk	High risk	
	Support for judgement		Randomized to receive one of two vaccines but no details of randomization process.	Double-blinded	234/237 completed the study. Results reported for varying number of subjects depending on assay (reported in figures/tables)	Figure 1 reports one infant only	

Pertussis

Author		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias) All outcomes	Incomplete outcome data (attrition bias) All outcomes	Selective reporting (reporting bias)	Other bias
Di Tommaso *et al* 1997	**Authors judgement**	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
	Support for judgement	Subjects recruited from those taking part in previous trials, unclear how many subjects there were in total in these previous trials.	Not randomised	Not blinded	All data appears to have been reported for each subject	All data appears to have been reported for each subject	All authors from company producing vaccine