What Would be Required to Prove Non-Target Effects of Vaccination ?

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What is being claimed by Aaby et al. ?

BCG vaccination reduces later mortality due to other disease.

Early measles vaccination reduces later mortality due to other diseases.

DTP vaccination increases later mortality and neutralizes the positive effects of BCG and measles vaccinations.

High titer measles vaccine increases mortality in girls, possibly due to concomitant DTP.

These effects are most influenced by last the vaccine received.

Observational Studies

DTP ↑ mortality

BCG ↓ mortality

Measles vaccine ↓ Mortality (Guinea-Bissau, Denmark)

Pentavalent vaccine ↑ mortality

OPV ↓ response to IPV

Controlled Trials

Measles vaccine ↓ mortality in absence of Vit A or IPV

BCG ↓ mortality in prematures

BPV ↓ beneficial effect of BCG

BCG ↑ cytokine production

OPV ↓ beneficial effect of BCG

Confirmatory Studies Outside Guinea Bissau

Prospective cohort in Uganda: BCG reduced mortality by 53%, 26% in 1-5 year olds

Measles-Yellow fever vaccine, but not DTP-Hib-Hep vaccine, reduced nasopharyngeal carriage of H. influenza and pneumococci

Naukabiru et al, BMC Public Health 2015
Bottomley et al. Emerg Themes Epid 2015

Possible Mechanisms for Vaccine Effects on Mortality

Positive effect:

- **Th1 response**
- **Th17 response**
- **Memory CD4 cells**
- **Cytokine response (IL17, IL22, IL 1β IL-6, TNFαl, IFNɣ**
- **NK cell memory**
- **Epigenetic programming of monocytes and NK cells**

Negative effect:

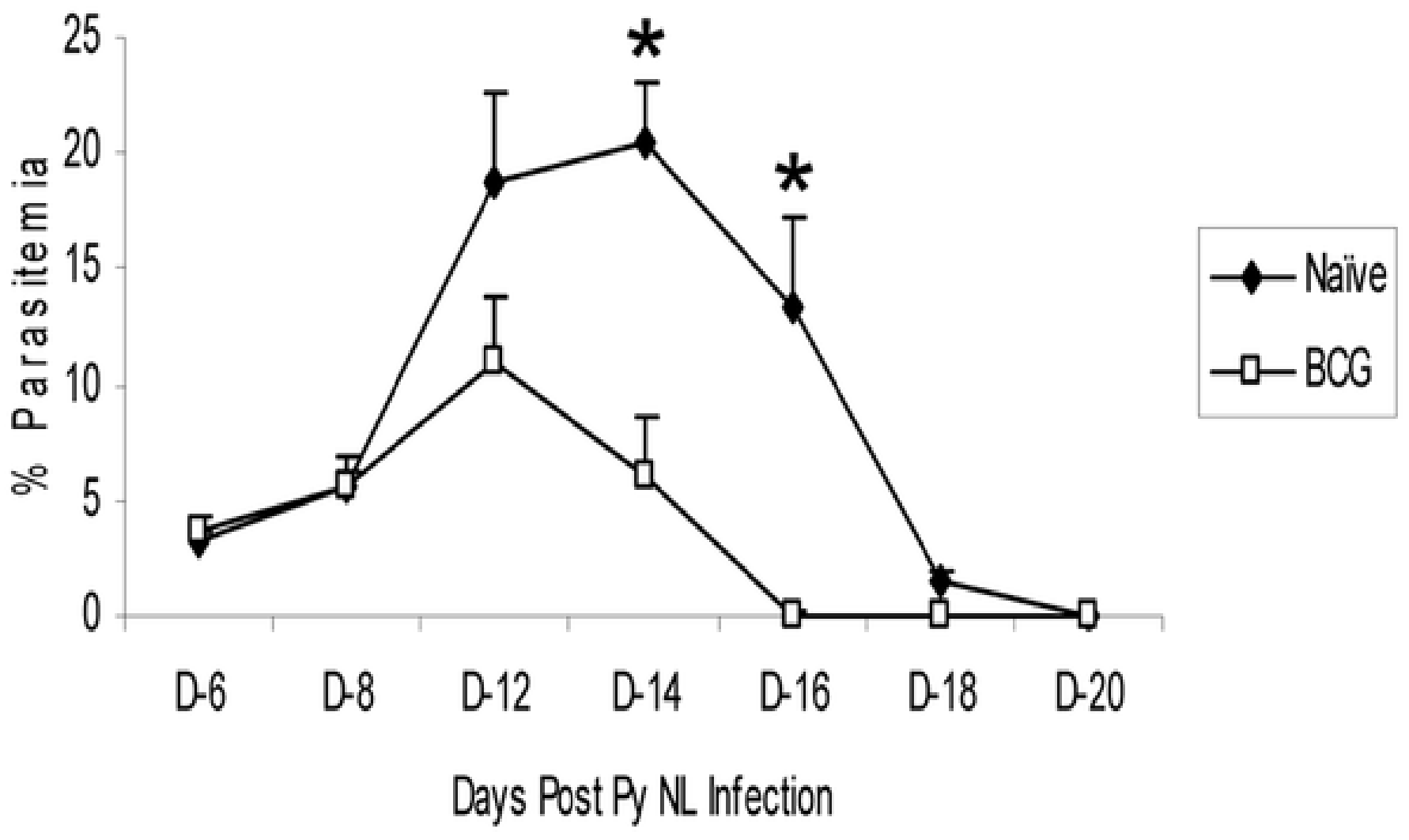
- **Th2 response**
- **CMV seropositivity**

Possible prophylactic effectiveness of enterovirus infection (LEV) and OPV in influenza and acute diseases

Group	Vaccinated with LEV or OPV			Internal control (no LEV)			Reduction of influenza incidence	
	total	developed disease		total	developed disease			
		n	%		n	%	ratio	%
I	11,799	418	3.55	8,218	486	5.91	1.7	41.2
II	40,678	6,305	15.50	18,880	5,456	28.90	1.9	46.4
III	99,575	5,634	5.71	40,419	7,163	17.71	3.1	67.7
Total	152,042	12,407	8.16	67,517	13,105	19.41	2.4	57.4

M. Voroshalova, Prog Med Virol, 1989

BCG vaccination confers partial protection against P. yoelii 17XNL infections in mice.



Parra M, Liu X, Derrick SC, Yang A, Tian J, et al. (2013) Molecular Analysis of Non-Specific Protection against Murine Malaria Induced by BCG Vaccination. PLoS ONE 8(7): e66115. doi:10.1371/journal.pone.0066115

Protection of mice against *Babesia* and *Plasmodium* with BCG

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Department of Zoology
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F.E. Cox

Nature, 1976

Comparison of GMC of IgG for each vaccine between BCG-immunised and non-BCG-immunised groups.

Vaccine	Geometric mean concentration (GMC) of IgG		p-value
	BCG-immunised (n = 56)	Non-BCG-immunised (n = 52)	
Pneumococcus			
Serotype 4	3.50	3.33	0.71
Serotype 6B	5.06	3.47	0.06
Serotype 9V	3.86	2.62	<0.01
Serotype 11A[a]	0.06	0.06	0.50
Serotype 14	9.77	8.69	0.43
Serotype 18C	3.09	2.32	0.04
Serotype 19F	3.69	3.14	0.18
Serotype 23F	3.80	3.32	0.50
Haemophilus influenzae type b	4.05	3.17	0.37
Tetanus	2.88	2.49	0.19
Hepatitis B	2908	4161	0.03

Ritz et al, Vaccine, 2013 June 26:31(30): 3098–3103

Sex-stratified effect of BCG strain on cytokine responses to tetanus toxoid (TT) and BCG scar frequency in Uganda

Cytokine	Sex (M/F)	Russia (*n* = 719)	Bulgaria (*n* = 508)	Denmark (*n* = 114)
		Geometric mean (pcg/ml)	Geometric mean (pcg/ml)	Geometric mean (pcg/ml)
IFN-γ	M	34.2	1.04	59.2
	F	23.9	25.23	115.3
IL-5	M	12.3	11.63	13.6
	F	10.0	6.94	19.7
IL-13	M	46.1	38.29	35.1
	F	40.4	26.28	74.9
IL-10	M	4.1	9.59	6.7
	F	2.7	7.70	15.9
BCG scar frequency (%)				
	M	182 (51.9%)	173 (65.3%)	53 (93.0%)
	F	170 (46.3%)	160 (65.8%)	53 (93.0%)

Anderson E.,Vaccine, 2012 March 9:30(12): 2083–2089

The NK Cell

Third lineage of lymphocytes

Important response to CMV infection, persisting for years.

NK cells increase after multiple infections.
e.g. vaccinia, influenza, hanta, particularly CMV

NK memory long-lived, depends on IL-15.

Transferred NK cells are active and protective.

Lactobacillus administration stimulates NK

Sun et al. EMBO J ., 2014
Foley et al, J. Immunol, 2012
Kawashira et al, Vaccine 2014

Other Non-Specific Activities

- Gamma-Delta T cells in the intestine
- Th1 + Th2 responses induced by BCG
  ↑ responses to OPV and Hepatitis B vaccines
- BCG increased CD4+ T cell responses to vaccinia
- Th2 response by acellular pertussis vaccines
- Candida programs monocytes against reinfection in the absence of B or T cells.

Sun et al, EMBO J, 2014
Ota et al, J immunol, 2002
Quintm et al, Cell 2012
Mathurin et al, J Virol, 2009

Another Factor to Consider ?

The Microbiome

TLR-5 Stimulation by flagellin on flagella influences responses to influenza vaccine

Gut overgrowth decreases response to oral cholera vaccine

Antibiotic treatment but also lactobacilli increases IgA response to rotavirus vaccine

Enteric bacteria enhance growth of norovirus

OPV immunogenicity decreased in malnourished infants

Oh, et al. Immunity 2014, Lages et al, JID, 1999, Uchiyama et al, JID 2014
Jones et al, Science 2014, Kandasany, Gut Microbes, 2014
Saleem et al, Vaccine 2015

Vaccines Affect Nasopharyngeal Carriage of Bacteria in Infants

	% Prevalence Before Vaccination	After vaccination
DTP-HibHepB		
pneumo	86.6	89.8
Hib	33.3	33.5
Staph	28.4	21.1
Measles - YF		
Pneumo	93.8	89.0
Hib	41.1	24.8
Staph	18.9	16.4

Bottomley, Emerg Themes Epid 2015

Interactions of Cytomegalovirus With the Immune System

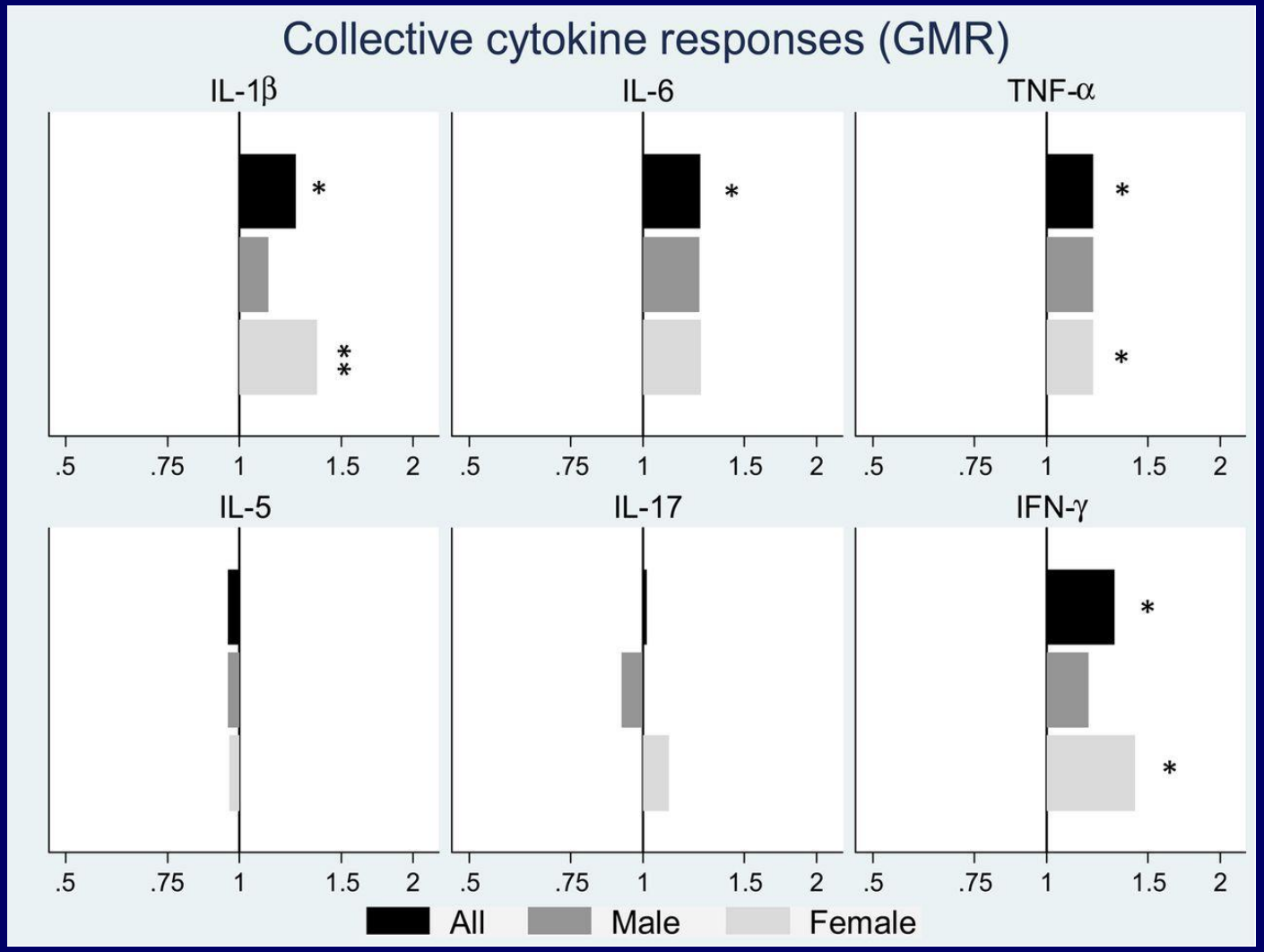
Induction of neutralizing , and ADCC antibodies
Induction of CD4+ T cell + CD8+ T cell responses to the virus

Also CMV

Produces proteins that downgrade antiviral T cell responses
Produces proteins that downgrade NK cell responses to first infection but increase NK memory
Produces gamma-delta T cells that increase antibody responses to donor grafts
Produces Treg cells that decrease inflammation
T cell responses to CMV may decrease responses to other antigens
Increases risk of coronary and cerebrovascular events in HIV infected (inflammation)

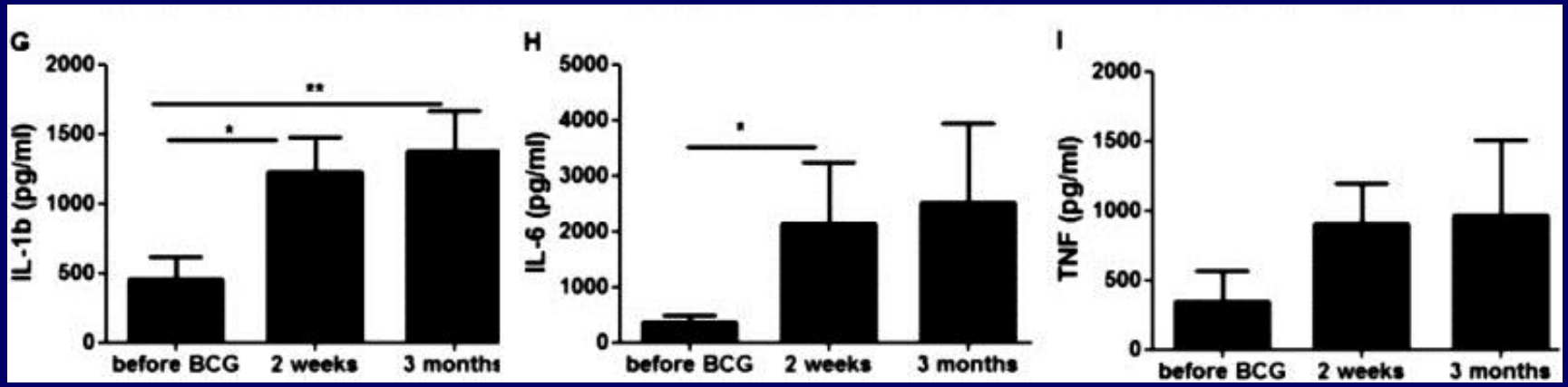
Terrazzini + Kern, F1000 Prime Reports 2014, Lichtner, JID, 2015
Couzi et al, Front Immunol, 2015
Boeckh et al, Biol Blood Marrow Transpl. 2015
Lee, Immunity, 2015

Geometric mean ratio (GMR) of in vitro cytokine production, comparing BCG-vaccinated to nonvaccinated overall and stratified by sex.



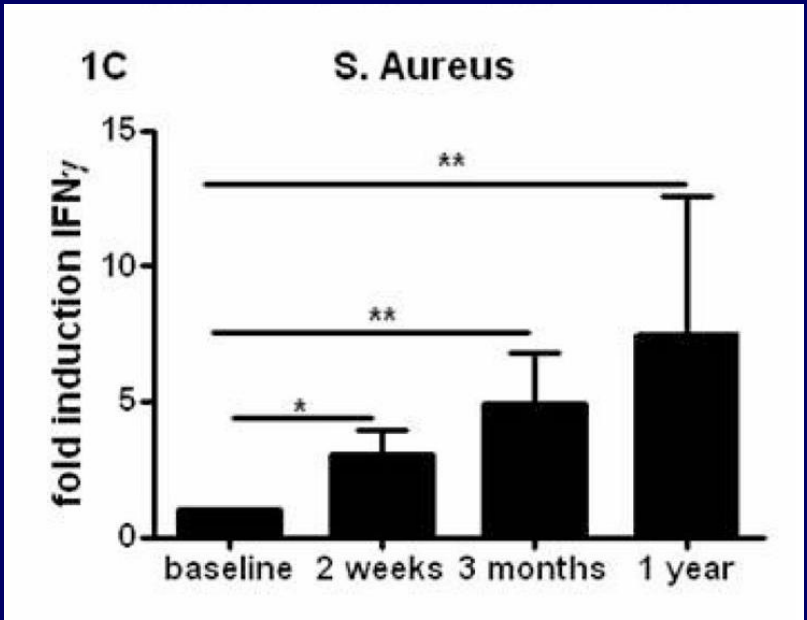
Kristoffer Jarlov Jensen et al. J Infect Dis. 2015;211:956-967

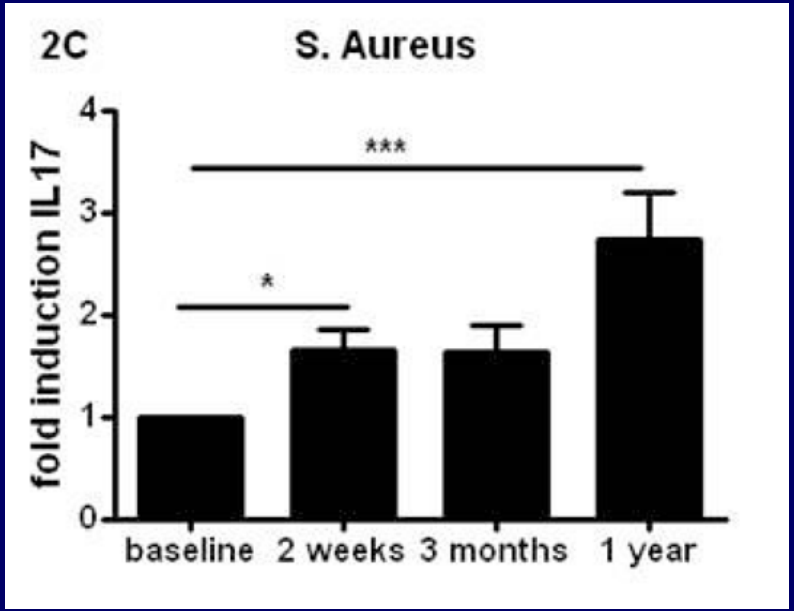
BCG enhances NK cell production of proinflammatory cytokines



Kleinnijenhuis, Clinical Immunology, 2014 December 155(2): 213–219

BCG Increases Th1 and Th17 Responses Against Heterologous Antigens





Kleinnijenhuis J., Journal of Innate Immun. 2014

Randomized Trial in Guinea Bissau

BCG vs BCG + OPV at birth

Combined vaccination ⬇ IFN and IL-5 Reponses to PPD at 2-6 weeks

KJ Jensen et al, Vaccine 2014

Need to Look Also at Children in Developed Countries

- **Mortality is easy to measure but insignificant in those countries**
- **Hospitalization and medical visits are better endpoint**
- **Immune markers should be influenced and measurable**
- **However, a study in Holland showed no effects on gender mortality after DTP-IPV vs. MMR**

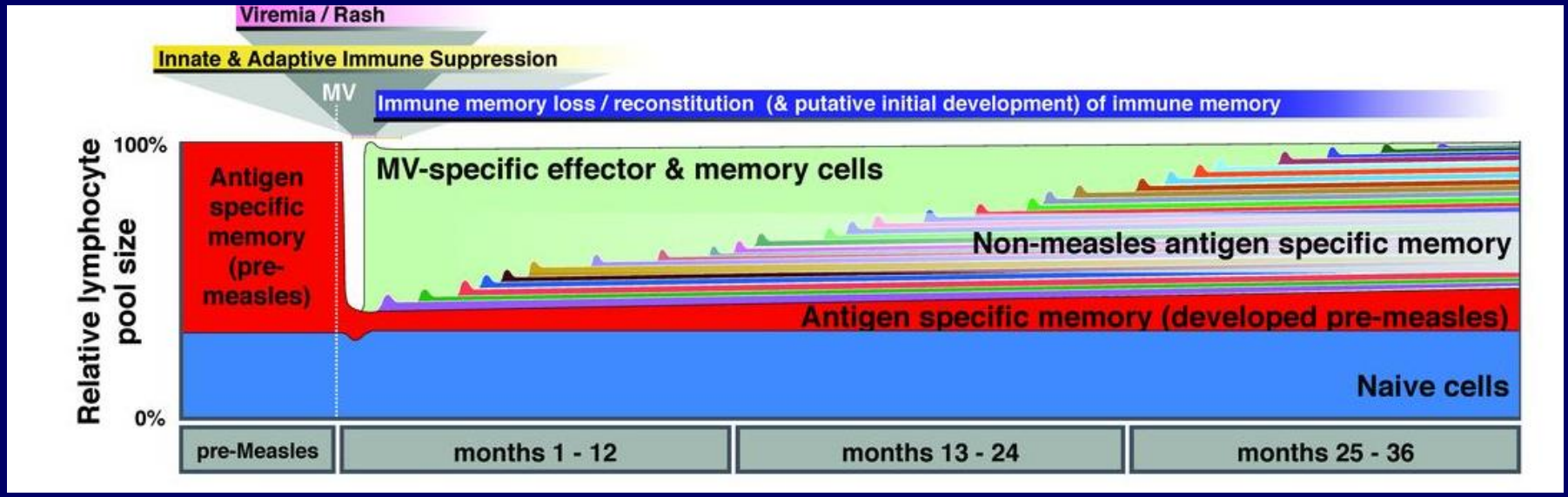
Schurink et al, BMC Infect Dis 2015

Measles and Immune Suppression

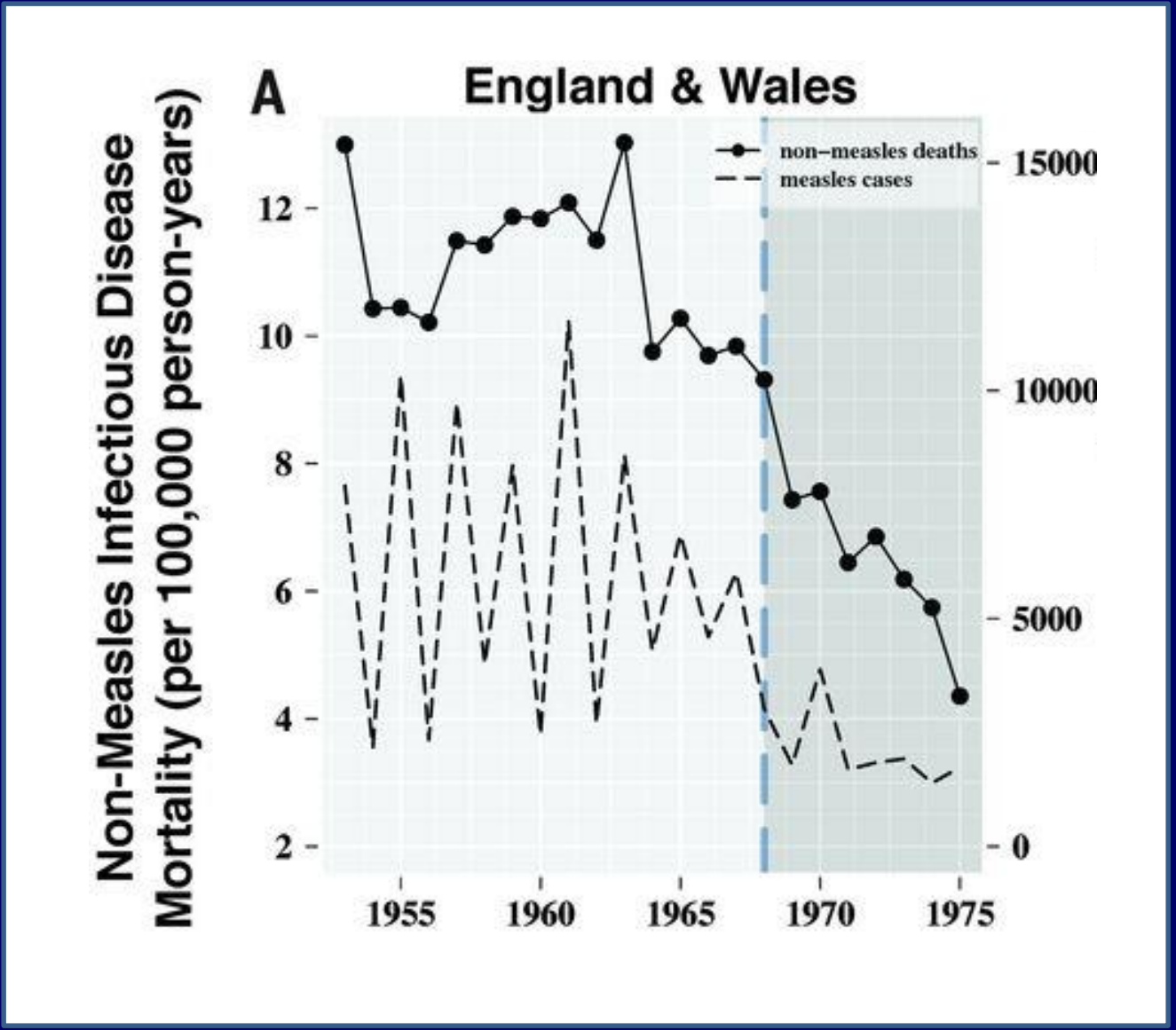
- **Measles virus replicates in dendritic cells, memory cells, memory T cells (CD150+) and follicular B cells**
- **Lymphopenia is transitory**
- **However, new lymphocytes are directed against measles and memory against other antigens is lost**

Devries et al, PLOS Pathogens 2012

Immune Memory Loss as Conceived by Mina et al, Science 2015



Decrease in Mortality Parallels Decrease in Measles



by Mina et al, Science 2015

- Until recently, claims for non-specific effects have been made based mainly on observations of mortality.

- However, epidemiology is mostly observational and sometimes conflicting (e.g. New Guinea vs. Guinea-Bissau)

- Only randomized epidemiology is convincing

- Proof must be buttressed by an immunological explanation.

- Mortality is a gross end point. Causes must be defined.

Lehman, Int J Epid, 2004

What Should be Studied?

- **Antibodies, particularly functionality**
- **Th cell orientation: 1, 2, 17**
- **Cytokine production and other innate immune responses**
- **NK activation**
- **CD8 T cells, effector and memory**
- **Coinfection with herpesvirus**

Bacillus Calmette-Guérin immunisation at birth and morbidity among Danish children: A prospective, randomised, clinical trial.

Lisbeth Marianne Thøstesen, Thomas Nørrelykke Nissen, Jesper Kjærgaard, Gitte Thybo Pihl, Nina Marie Birk, Christine Stabell Bennd, GormGreisen, Poul-Erik Kofoeda, Ole Pryds, Henrik Ravn, Dorthe Lisbeth Jeppesen, Peter Aaby , Lone Graff Stensballe

Methods: The Danish Calmette Study is amulticentre randomised clinical trial conducted between October 2012 and November 2015.Within the first 7 days of life, infants were randomly assigned to intra-dermal vaccination with BCG or no intervention. At 3 and 13 months of age structured telephone interviews and clinical examinations of the children were conducted. In a subgroup of children blood samples were drawn and stool samples collected at age 4 days, 3 and 13 months. Thymus index was assessed by ultrasound in a subgroup at randomisation and at 3 months. The primary study outcome is hospitalisation within the first 15 months of life as assessed in Danish health registers. Secondary outcomes include infectious disease hospitalisations, wheezing, eczema, use of prescribed medication, growth, development, thymus index, T- and B-cell subpopulations assessed by flow cytometry, in vitro cytokine responses and specific antibody responses to other vaccines. Adverse reactions were registered.

In Summary

Non-specific effects exist and are a valid field of study.

Emphasis must now be put on immunological mechanisms.

Demonstration of similar effects in developed countries, even without mortality, would help validate findings in developing countries. However, more controlled studies are needed in developing countries.

The work of Aaby et al has been justified, although much needs to be clarified.

There is nothing more difficult

to take in hand, more perilous to conduct,

or more uncertain in its success,

than to take the lead in the

introduction of a new order of things.

Niccolo Machiavelli