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Title: Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood.

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[Abstract]
Ecological factors are important determinants of the development and function of anti-pathogen defences. Inflammation is a central part of innate immunity, but the developmental factors that shape the regulation of inflammation are not known. We test the hypothesis that microbial exposures in infancy are associated with high sensitivity C-reactive protein (CRP) in adulthood using prospective data from a birth cohort in the Philippines (n = 1461). Lower birth weight was associated with increased CRP, consistent with a role for inflammation in the widely documented inverse relationship between birth weight and adult cardiovascular diseases. In addition, higher levels of microbial exposure in infancy were associated with lower CRP. These associations were independent of socioeconomic status, measures of current body fat and other health behaviours. We conclude that measures of microbial exposure and nutrition during the pre-natal and early post-natal periods are important predictors of CRP concentration in young adulthood. We speculate that the development of anti-inflammatory regulatory networks in response to early microbial exposure represents plasticity in the development of anti-pathogen defences, and that this process may help explain the low CRP concentrations in this population.

Keywords: inflammation; infectious disease; cardiovascular disease; developmental

Early:
Later:
Inflammation:
CRP: a key biomarker of inflammation

Hygienic environment ⇒

Infectious exposure ⇒

Health status of later in life?

1. Introduction

1-1. Early"bad" environment → risk for diseases in adulthood

-Death rates during infancy/childhood ↑＝＝ life expectancy ↓(birth cohort based analysis)
(Kermack et al. 1934: Death rates in Great Britain and Sweden: some general regularities and their significance. Lancet 226, 698–703.)

- How “bad”?
  A. Under nutrition → risk for cardiovascular and metabolic diseases
  (developmental modifications to physiological systems: Barker 1994; Gluckman et al. 2008).
  B. Early infectious exposure → chronic inflammatory pathways → morbidity/mortality
  Hypothesized by [Finch, C. E. & Crimmins, E. M. 2004 Inflammatory exposure and historical changes in human life-spans. Science 305, 1736–1739] and [Crimmins, E. M. & Finch, C. E. 2006 Infection, inflammation, height, and longevity. Proc. Natl Acad. Sci. USA 103, 498–503]

  CRP = incident CVD (Ridker et al. 1998), type 2 diabetes (Pradhan et al. 2001), the metabolic syndrome (Ridker et al. 2003), late-life disability (Kuo et al. 2006) and mortality (Jenny et al. 2007) in older adults.

1-2. Early infectious exposure → stimulate the development of immune system (Sheldon & Verhulst 1996; Schulenburg et al. 2009)

Well developed immune system: usually CRP=low but elevated when necessary
Poorly developed immune system: usually CRP=high (“immune dysregulation”**)

Immune dysregulation= e.g., atopic diseases, CVD, DM…

1-3. Objectives: to test the hypothesis that microbial and infectious exposures in infancy are associated with CRP in young adulthood using data from a large prospective birth cohort study in the Philippines.

2. Materials and Methods

2-1. Participants and data collection
-CLHNS (Cebu Longitudinal Health and Nutrition Survey)
-Single-stage cluster sampling →17 urban and 16 rural neighborhoods:
all pregnant women in the targeted neighborhoods = 3327 (1983)
-1885 children and 1461 women could be studied in 2005
-Bimonthly interview/observation for the first 2 years

-Evaluation of selection bias:
Original cohort = people who could be studied in 2005 + those dropped out

2-2. CRP analysis: dependent analysis
-blood plasma, a high sensitivity immunoturbidimetric method (Synchron LX20, lower detection limit: 0.1 mg/l)

*Categorized variable: >=0.7mg/l (a top-tertile) and 0.7mg/l> (lower two tertiles): logistic reg
*Continuous variable: Log (CRP) ---left censored: Tobit regr

2-3. Independent variables
<variables for the first 2 years>
-Infectious morbidity: (24 visits /year) ×2 years =48 interviews = diarrhea/respiratory symptoms
Variable: Number of cases in 2-12 months and that in 14-24 months
- Exposure to infectious microbes (household level)
*household density (number of persons/number of rooms),
* faecal contamination of the yard outside the home (0=no faeces, 4=heavy contamination)
*type of toilet (no toilet or pit versus flush/water sealed)
*source of drinking water (closed sources versus open sources)
*Exposure to animal feaces (total number of observations infant crowing and animal

presence/48 observations)
-Dry season: February-April, Wet season: June-October
-Prenatal/postnatal environment: birth weight, gestational age, parity, growth during the first 2 years, duration of breast feeding

<Variables during adulthoods>
-Symptoms of infection
-Anthropometric measurements, health behaviors (smoking, drinking, OC use), SES (income, assets, education)
-household pathogen exposure variable: cleanliness of the food preparation area (0-2), means of garbage disposal (0-2), presence of excrement near the house (0-2), level of garbage and excrement present in the neighborhood surrounding the household (0-2)

2-4. Analysis
- Individuals with CRP>10mg/l (n=52, 3.3%)were removed: results of acute inflammatory processes (American Heart Association and the Centers for Disease Control and Prevention; Pearson et al. 2003)

3. Results

Table 1. Descriptive statistics for female and male participants. Mean (s.d.) values are presented for continuous variables ($n = 1461$).

	female ($n = 672$)	male ($n = 862$)	total ($n = 1534$)
assessed in infancy			
birth weight (kg)	2.991 (0.402)	3.029 (0.433)	3.012 (0.420)
gestation length (weeks)	39.2 (2.5)	38.8 (2.4)	39.0 (2.5)
weight gain, 1st year (kg)	4.678 (0.860)	5.217 (0.934)	4.978 (0.945)
mother's education (years)	7.3 (3.6)	7.5 (3.7)	7.4 (3.6)
household income (pesos)	278.1 (411.7)	286.3 (539.0)	282.7 (486.8)
duration exclusive breastfeeding (days)	60.2 (38.9)	57.0 (39.5)	58.4 (39.3)
assessed in adulthood			
age (years)	20.9 (0.3)	20.9 (0.3)	20.9 (0.3)
education (years)	11.6 (3.3)	10.5 (3.8)	10.9 (3.6)
waist circumference (cm)	68.1 (7.7)	72.1 (7.5)	70.3 (7.8)
sum of three skinfold measures (mm)	62.3 (19.6)	37.6 (18.1)	48.4 (22.4)
symptoms of infection (%)	14.6	13.7	14.1
oral contraceptive use (%)	3.7		
CRP (median, 25th, 75th percentile)	0.2 (0.1, 0.9)	0.3 (0.1, 0.9)	0.2 (0.1, 0.9)

Table 3. Results of maximum-likelihood logistic regression models predicting the probability of CRP in the top tertile (≥ 0.7 mg l^{-1}), excluding individuals with CRP > 10 mg l^{-1} ($n = 1409$).[a]

	unadjusted		model 1		model 2	
	OR	95% CI	OR	95% CI	OR	95% CI
diarrhoea, 1st year (no. episodes)	0.94	0.84, 1.05	0.98	0.87, 1.10	0.98	0.87, 1.11
diarrhoea, 2nd year	0.90*	0.81, 0.99	0.90†	0.81, 1.00	0.89*	0.80, 0.99
resp. infect., 1st year	0.97	0.89, 1.06	0.97	0.88, 1.06	1.00	0.90, 1.10
resp. infect., 2nd year	1.02	0.94, 1.11	1.05	0.96, 1.15	1.00	0.91, 1.10
faeces near house (0–4)	1.00	0.82, 1.21	1.04	0.85, 1.28	1.02	0.82, 1.26
animal faeces in house (no. intervals)	0.86**	0.78, 0.94	0.86**	0.78, 0.94	0.87**	0.79, 0.96
open water source (0, 1)	0.90	0.63, 1.28	0.87	0.59, 1.26	0.91	0.60, 1.38
flush toilet (0, 1)	0.99	0.79, 1.25	0.95	0.74, 1.21	0.94	0.72, 1.24
household density (persons/room)	0.98	0.91, 1.06	0.98	0.90, 1.06	0.96	0.89, 1.05
dry season birth (0, 1)	0.70*	0.52, 0.94	0.70*	0.51, 0.94	0.67*	0.49, 0.92
birth weight (kg)	0.78†	0.59, 1.03	0.77†	0.59, 1.02	0.71*	0.53, 0.95

[a]Model 1 includes variables listed in table 3 and adjusts for gender. Model 2 includes these variables, but also adjusts for maternal education, household income at birth, current household income, current waist circumference, skinfold thickness, oral contraceptive use, symptoms of infection at the time of blood collection and household pathogen exposure.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
†$p < 0.10$.

Table 2. Bivariate associations between elevated CRP in young adulthood and measures of infectious exposures in infancy (excluding individuals with CRP $> 10\ mg\ l^{-1}$). Mean (s.d.) values are presented for continuous variables; percentages are presented for categorical variables. Two-sample t-tests (continuous variables) and Pearson χ^2-tests (categorical variables) were used to evaluate differences between groups with CRP < 0.7 and CRP $\geq 7\ mg\ l^{-1}$.

	CRP $< 0.7\ mg\ l^{-1}$	CRP $\geq 0.7\ mg\ l^{-1}$
diarrhoea, 1st year (no. episodes)	1.12 (1.10)	1.05 (0.99)
diarrhoea, 2nd year	1.20 (1.20)	1.06 (1.14)*
respiratory infection, 1st year (no. episodes)	4.37 (1.35)	4.31 (1.33)
respiratory infection, 2nd year	4.33 (1.42)	4.37 (1.40)
level of faecal contamination near house (0–4)	0.87 (0.99)	0.85 (1.01)
animal faeces in house, 6–12 months (no. bimonthly intervals)	1.33 (1.30)	1.08 (1.20)**
open water source (%)	89.0	87.9
flush toilet (%)	45.1	44.8
household density (persons/room)	2.55 (1.55)	2.50 (1.48)
dry season birth (%)	22.3	16.8*

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

More diarrhoea in the second year
→ lower CRP
More exposure to animal feaces
→ lower CRP
Born in dry season
→ lower CRP
(Table 2)

More diarrhoea in the second year, more exposure to animal feaces in house, dry season birth, lower birthweight
→ lower CRP
(Table 3)

More exposure to animal feaces in house, and lower birthweight
→ lower CRP
(Table 4)

Fig 1 and Fig 2: Table 3, Model 2

Reanalysis by including the individuals with CRP>10mg/l did not show the different tendency.

Table 4. Results of Tobit regression models predicting log-transformed CRP, excluding individuals with CRP $> 10\ mg\ l^{-1}$ ($n = 1409$).[a]

	unadjusted		model 1		model 2	
	B	s.e.	B	s.e.	B	s.e.
diarrhoea, 1st year (no. episodes)	−0.028	0.024	−0.021	0.025	−0.018	0.024
diarrhoea, 2nd year	−0.029	0.021	−0.029	0.023	−0.025	0.021
resp. infect., 1st year	−0.005	0.019	−0.009	0.020	0	0.019
resp. infect., 2nd year	0.017	0.018	0.024	0.019	0.006	0.018
faeces near house (0–4)	0.006	0.043	0.019	0.044	0.011	0.042
animal faeces in house (no. intervals)	−0.075***	0.020	−0.073***	0.020	−0.063**	0.019
open water source (0, 1)	−0.074	0.079	−0.099	0.081	−0.061	0.081
flush toilet (0, 1)	0.016	0.051	−0.004	0.053	−0.007	0.053
household density (persons/room)	0.011	0.016	0.011	0.017	0.004	0.016
dry season birth (0, 1)	−0.083	0.063	−0.078	0.063	−0.082	0.060
birth weight (kg)	−0.137*	0.060	−0.142*	0.060	−0.171**	0.057
constant			−0.158	0.232	−0.822†	0.469

[a]Model 1 includes variables listed in table 4 and adjusts for gender. Model 2 includes these variables, but also adjusts for maternal education, household income at birth, current household income, current waist circumference, skinfold thickness, oral contraceptive use, symptoms of infection at the time of blood collection and household pathogen exposure.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
†$p < 0.10$.

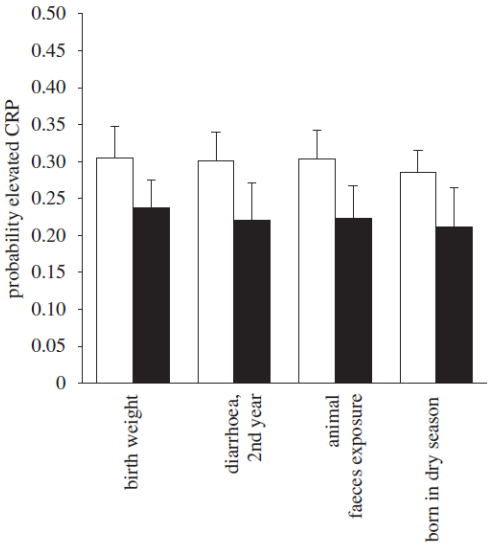

Figure 1. Probability of elevated CRP in relation to birth weight and pathogen exposure in infancy. Note: values are predicted probability of CRP in top tertile for the entire sample (excluding individuals with CRP $\geq 10\ \mathrm{mg\,l^{-1}}$) based on coefficients from table 3, model 2, with upper bound of 95% CI. Low and high values for predictors were set as follows: birth weight (2.5 and 3.5 kg); diarrhoea, 2nd year (0, 3 or more episodes); animal faeces exposure (0, 3 or more intervals); born in dry season (no, yes). Original values were retained for other covariates. Unshaded box, low/no; shaded box, high/yes.

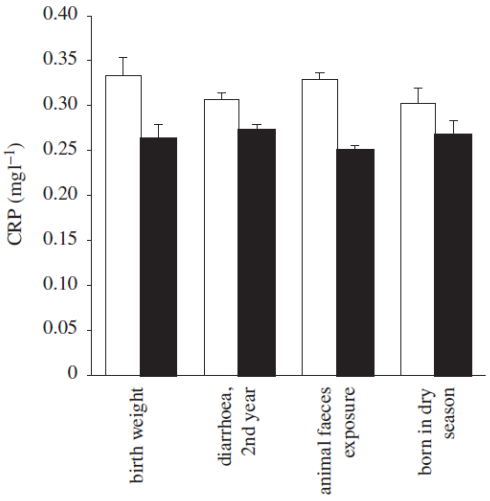

Figure 2. Concentration of CRP in relation to birth weight and pathogen exposures in infancy. Note: values are predicted concentration of CRP in the entire sample (excluding individuals with CRP $\geq 10\ \mathrm{mg\,l^{-1}}$) based on coefficients from table 4, model 2. Predicted values are presented with the standard error of the estimate. Low and high values for predictors were set as follows: birth weight (2.5 and 3.5 kg); diarrhoea, 2nd year (0, 3 or more episodes); animal faeces exposure (0, 3 or more intervals); born in dry season (no, yes); adult waist circumference (± 1 s.d.); adult skinfold thickness (± 1 s.d.). Original values were retained for other covariates. Unshaded box, low/no; shaded box, high/yes.

4. Discussion

4-1. Summary of findings

*Microbial exposure early in life (as evidenced by exposure to animal faeces in house, diarrhea (2nd year), dry season birth) was negatively associated with CRP production in adulthood

4-2. Contradiction with hypothesis 1.1 (above)

*timing of CRP evaluation

*Type of pathogenic exposure in Philippine in the 1980s and that in the previous studies

*timing of pathogenic exposure (2 years<)

4-3. “Hygiene hypothesis” or “old friends hypothesis”:

-chronic exposure to harmless microbes common throughout human evolutionary history is critical to immune development, and the absence of such exposure is the key factor leading to immune dysregulation (Rook et al. 2004, 2009):

*exposure to animal faeces in house
> direct measures of pathogenic infections (e.g., household density)

*Possible mechanisms: Epigenetic modifications to regulatory genes related to CRP are a potential mechanism linking early exposures with adult phenotypes that are worthy of further investigation (Bateson et al. 2004; Waterland & Michels 2007).

4-4. Comparison

US: CRP=2 mg/l (median) for 17 years and older, 0.9 mg/l for 20-29 years men.
Scotland/German: CRP=0.81-1.25 mg/l (geometric mean) for 25–34-years men/women
Cf. 0.2 mg/l (the present study); the amount of fat mass can not fully explain the difference.

4-5. Low birth weight and higher CRP in later life, low birth weight and CVD, DM, hypertention..

4-6. Limitation: single measurement of CRP, proxy measure of exposure, migration.