

**Can worms defend our hearts?**

**Chronic *Opisthorchis felineus* (helminthic)  
infection attenuates atherosclerosis  
– An autopsy study**

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# Introduction

In 1947, Norman Stoll estimated that among the 2.2 billion world population (in 1947)

- 644 million individuals were infected with *Ascaris lumbricoides* (30% prevalence)
- 355 million with *Trichuris trichiura* (16%)
- 457 million (21%) with *Necator americanus* and *Ancylostoma duodenale*.

Stoll NR. This wormy world. J Parasitol 1947;33:1–18.

# Possible immune and infectious basis of atherosclerosis

The established risk factors for atherosclerosis fail to fully explain the extent and severity of coronary artery diseases in 50% of patients, even in the developed countries.

The infectious theory of atherosclerosis has built up from the pioneering observations of Fabricant et al.

So far, one virus (*cytomegalovirus*) and two bacteria (*Chlamydia pneumoniae* and *Helicobacter pylori*) have been implicated in human atherosclerosis, based upon experimental, epidemiologic, or pathologic evidence.

These potential contributions have yet to be established beyond reasonable doubt .

# Objective

Previously, we proposed a hypothesis that chronic helminthic infection may have beneficial effects on the development of atherosclerosis.

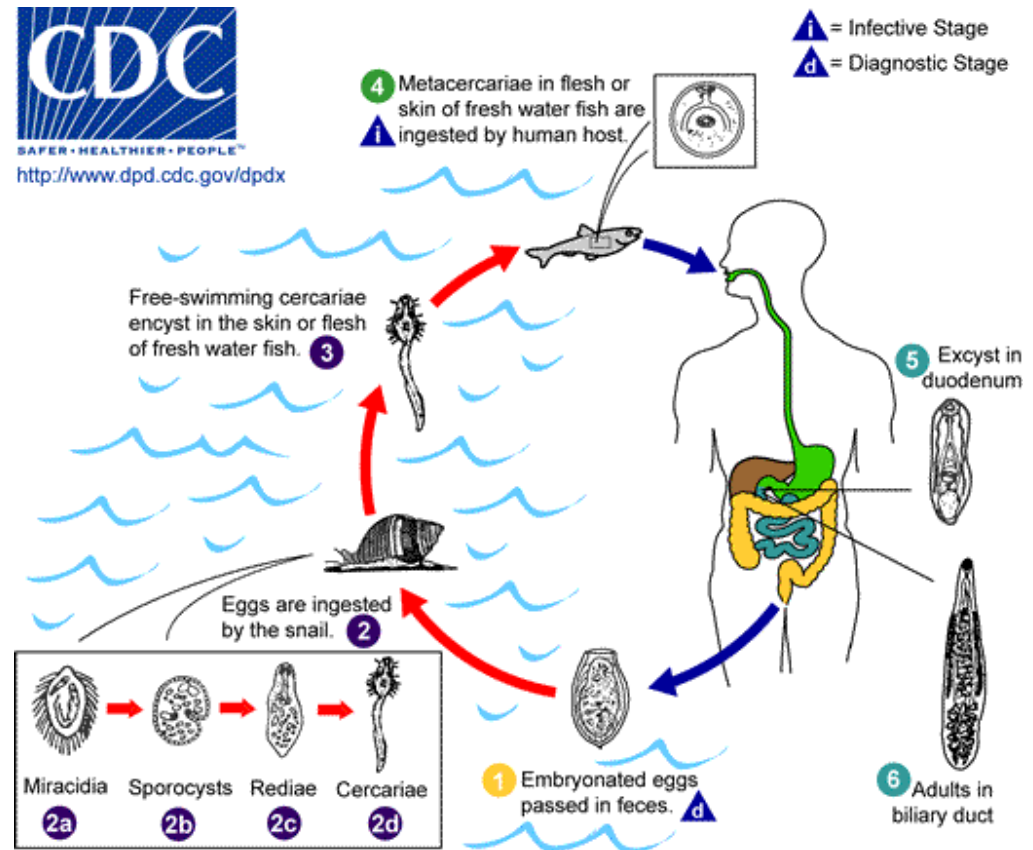
Magen E, Borkow G, Bentwich Z, Mishal J, Scharf S. Can worms defend our hearts? Chronic helminthic infections may attenuate the development of cardiovascular diseases. Med Hypotheses. 2005;64(5):904-9.

# **The aim of the study**

The aim of the study was to investigate an association between chronic helminthic infection with aortic atherosclerosis in humans.

# *O. felineus* chronic helminthic infection

Most individuals with mild to moderate *O. felineus* chronic helminthic infection (CHI) show no significant signs or symptoms of disease compared with uninfected matched control groups (Bychkov et al., 1990).



# Endemic zone for *O. felineus* infection

- Khanty-Mansiisk, an oil boom-town and the administrative center of Khanty-Mansi Autonomous Okrug, Russia, is located on the eastern bank of the Irtysh River.



- Because river fish carrying the helminth *Opisthorchis felineus* is a prevalent source of food here, this area constitutes a very large endemic zone for *O. felineus* infection, reaching 57.6 % in humans and 70.8 % in animals.

Zelia, O.P., Zavoi'kin, V.D., Sergeeva, M.N. , The characteristics of the circulation of the causative agent of opisthorchiasis in the tributaries of the Ob. *Med. Parazitol.* 1990; 4, 22–25.

# Epidemiological surveys

- Older small-scale studies reported relatively low prevalence and incidence of CVD in this endemic region.
- While statistics for the entire Union of Soviet Socialist Republics (USSR) showed deteriorating male life expectancy in the period 1970–1989, due mainly to increased deaths from CVD.

Puzyrev, V.P., Lemza, S.V. Structure of ischemic heart disease risk factors in the North Khanty population of western Siberia. *Genet. Epidemiol.* 1990; 7, 255–259.

Puzyrev, V.P, Galaktionov, O.K, Efimov, V.M, Saliukov V.B, Ostretsova O.A. Multifaceted analysis of the interactions of morphophysiologic signs and ischemic changes (a population study). *Kardiologiia* , 1989; 29, 75–77



# Material and methods

- An autopsy series of people subjected to a medicolegal autopsy was studied in order to investigate the association of CHI with autopsy-confirmed aortic atherosclerosis (AA).
- Indications for an autopsy were out-of-hospital death of a previously healthy person due to accidental death, suicide, accidental violence or other traumatic injury.
- Autopsies are performed on 78% of all deaths of people <65 years old in this area.

# Material and methods

- The cadavers were brought to the mortuary within 12 h of death for the autopsy examination.
- The bodies were refrigerated and forensic autopsies were performed by certified pathologists from the Division of Forensic Medicine, Tyumen Medical Academy, Russian Federation within a day after receipt of the bodies.

# Material and methods

- A family history of heart disease, smoking, hypertension or diabetes, and serum total cholesterol levels of all patients examined was elicited.
- Pertinent clinical information and autopsy findings were recorded on the standardized basic data form.

# Material and methods

The examined groups were characterized by:

- **The number examined:** the autopsy series included 319 consecutive cases of subjects (280 (87.8%) males and 39 (12.2%) females) aged 20–71 years old subjected to a medico-legal autopsy.
- **Age groups:** subjects were divided into five age groups: (i) 20–29, (ii) 30–39, (iii) 40–49, (iv) 50–59 and (v) >60 years old.
- **Level of invasion:** *O. felineus*-infected subjects were further divided into three sub-categories, depending on the worm burden: mild (<100 worms), moderate (100–500 worms) and severe (>500 worms).

# Measurements of area of AA lesions

The areas of the different types of atherosclerotic lesions in the thoracic and abdominal aortas were measured.

The characterization of AA was based on the protocols of two international studies:

1. the International Atherosclerosis Project, (Guzman et al., 1968)
2. the WHO Study Group in Europe (Uemura et al., 1964).

# Measurements of area of AA lesions

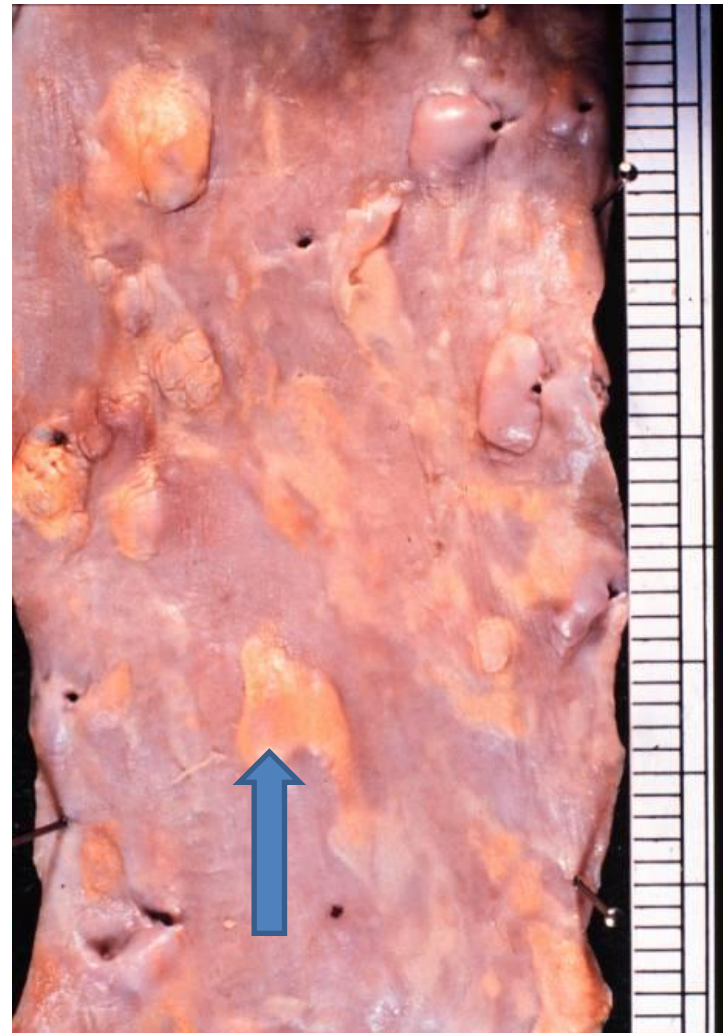
The vessel wall was stained with the **Sudan IV fat-staining method**.

Stained areas that showed no other type of underlying change were classified as **fatty streaks**.



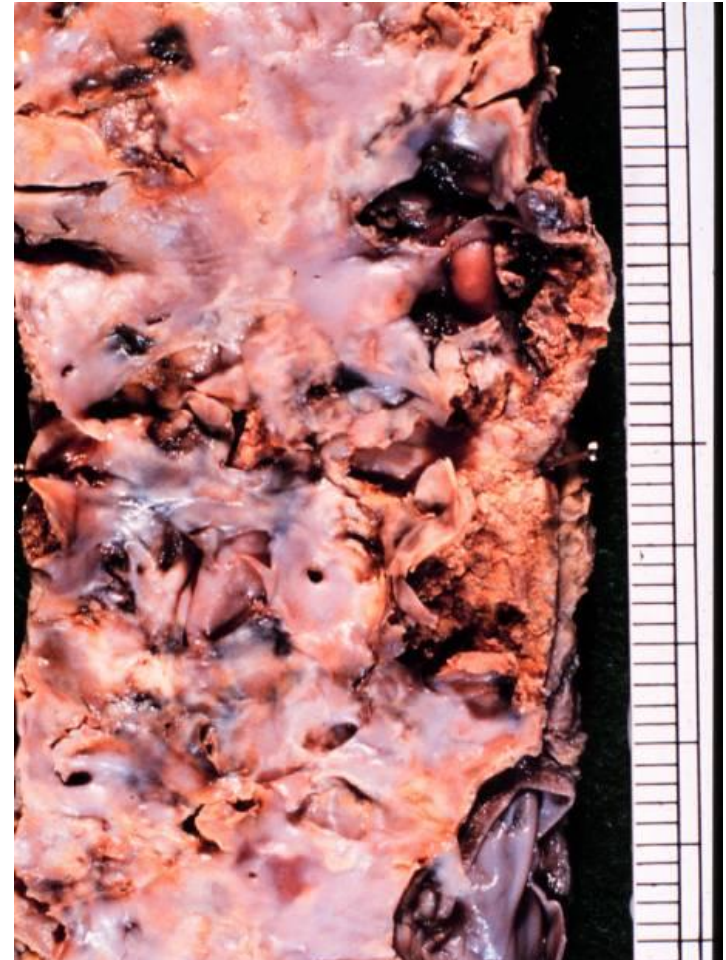
# Measurements of area of AA lesions

Elevated plaques exhibiting no ulceration or thrombosis were considered **fibrotic plaques**



# Measurements of area of AA lesions

While those with ulceration or thrombosis were classified as **complicated lesions**.





# Measurements of area of AA lesions

- The areas exhibiting fatty streaks, fibrotic plaques and complicated lesions were measured by standard planimetry (Uemura et al., 1964) by the digital planimeter.

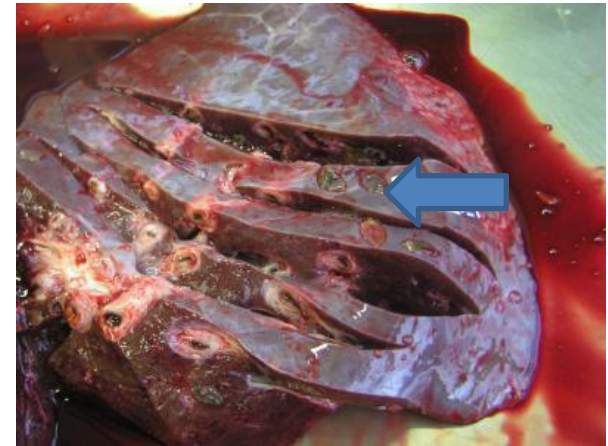


- A single lesion was measured in square millimeters and the areas of different types of lesions were expressed in percentages by dividing the lesion area by the total area of the artery wall and multiplying by 100%.

# Parasitological examination

- The liver was removed and weighed after completion of the forensic examination.
- In order to assess *O. felineus* worm burden, each liver was investigated for the presence of parasites according to Sithithaworn et al. (1991).

Briefly, the liver was cut cross-wise into pieces approximately 1 cm thick; these pieces were squeezed gently to release worms from the bile ducts into normal saline. The worms were then sedimented and counted.



Sithithaworn, P., Tesana, S., Pipitgool, V., Kaewkes, S., Pairojkul, C., Sripa, B., Paupairoj, A., Thaiklar, K., 1991. Relationship between faecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. *Parasitology* 102, 277–281

# Total cholesterol measurements

Blood from the heart cavities was sampled within 24 h post-mortem.

The samples were stored at -20 C until they were shipped to the central laboratory, where they were analyzed within 2 days of collection.

# Characteristics of *O. felineus*-infected subjects and controls

Clinical and laboratory characteristics of cardiovascular disease risk factors.

Age (years)	Variables	Mild <i>Opisthorchis felineus</i> burden	Moderate <i>O. felineus</i> burden	Severe <i>O. felineus</i> burden	Non-infected controls
20–29	Sex M/F (total)	11/1 ( <i>n</i> = 12)	12/3 ( <i>n</i> = 15)	15/1 ( <i>n</i> = 16)	10/2 ( <i>n</i> = 12)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.4 ± 3.8	23.9 ± 3.7	23.1 ± 3.4	24.3 ± 3.5
	Total cholesterol (mg/dL) <sup>a</sup>	178.1 ± 18.6	177.6 ± 22.3	168.8 ± 18.4 <sup>c</sup>	186.1 ± 9.1
	Hypertension, <i>n</i> (%)	0	0	0	0
	Diabetes, <i>n</i> (%)	0	0	0	0
	Smoking, <i>n</i> (%)	7 (58.3%)	9 (60%)	7 (43.7%)	5 (41.6%)
	Family history of CAD, <i>n</i> (%)	4 (33.3%)	4 (26.6%)	3 (18.7%)	3 (25%)
30–39	Sex M/F (total)	12/2 ( <i>n</i> = 14)	17/3 ( <i>n</i> = 20)	15/2 ( <i>n</i> = 17)	17/1 ( <i>n</i> = 18)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.7 ± 3.5	24.5 ± 3.9	23.4 ± 3.1	25.2 ± 4.3
	Total cholesterol (mg/dL) <sup>a</sup>	183.2 ± 27.7	181.5 ± 28.5	167.1 ± 23.5 <sup>c</sup>	192.6 ± 21.9
	Hypertension, <i>n</i> (%)	0	1 (5%)	0	0
	Diabetes, <i>n</i> (%)	0	0	0	0
	Smoking, <i>n</i> (%)	5 (35.7%)	12 (60%)	6 (35.2%)	7 (38.9%)
	Family history of CAD, <i>n</i> (%)	4 (28.5%)	3 (15%)	4 (23.5%)	3 (16.7%)
40–49	Sex M/F (total)	14/0 ( <i>n</i> = 14)	15/1 ( <i>n</i> = 16)	15/3 ( <i>n</i> = 18)	14/1 ( <i>n</i> = 15)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.6 ± 4.1	24.2 ± 3.9	24.5 ± 3.2	25.1 ± 4.3
	Total cholesterol (mg/dL) <sup>a</sup>	184.8 ± 29.2	181.9 ± 19.2 <sup>b</sup>	163.3 ± 24.1 <sup>d</sup>	201.3 ± 16.6
	Hypertension, <i>n</i> (%)	1 (7.1%)	2 (12.5%)	1 (5.6%)	1 (6.7%)
	Diabetes, <i>n</i> (%)	1 (7.1%)	0	0	0
	Smoking, <i>n</i> (%)	5 (35.7%)	7 (43.8%)	9 (50%)	7 (46.7%)
	Family history of CAD, <i>n</i> (%)	2 (14.3%)	3 (18.8%)	1 (5.6%)	4 (26.7%)

BMI, body mass index; CAD, coronary artery disease.

<sup>a</sup> Values are mean ± S.D.

<sup>b</sup> *P* < 0.05.

<sup>c</sup> *P* < 0.01.

<sup>d</sup> *P* < 0.001.

# Characteristics of *O. felinus*-infected subjects and controls

**Table 1**

Clinical and laboratory characteristics of cardiovascular disease risk factors.

Age (years)	Variables	Mild <i>Opisthorchis felinus</i> burden	Moderate <i>O. felinus</i> burden	Severe <i>O. felinus</i> burden	Non-infected controls
50–59	Sex M/F (total)	15/3 ( <i>n</i> = 18)	9/1 ( <i>n</i> = 10)	17/2 ( <i>n</i> = 19)	11/3 ( <i>n</i> = 14)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.4 ± 3.8	25.3 ± 3.1	24.2 ± 3.5	25.6 ± 4.2
	Total cholesterol (mg/dL) <sup>a</sup>	191.4 ± 29.6	187.6 ± 16.1	174.3 ± 26.3 <sup>d</sup>	210.2 ± 20.1
	Hypertension, <i>n</i> (%)	3 (16.7%)	4 (40%)	6 (31.6%)	4 (28.6%)
	Diabetes, <i>n</i> (%)	2 (11.1%)	1 (10%)	3 (15.8%)	2 (14.3%)
	Smoking, <i>n</i> (%)	8 (44.4%)	6 (60%)	6 (31.6%)	9 (64.3%)
	Family history of CAD, <i>n</i> (%)	2 (11.1%)	2 (20%)	1 (5.3%)	3 (21.4%)
>60	Sex M/F (total)	15/2 ( <i>n</i> = 17)	16/0 ( <i>n</i> = 16)	17/3 ( <i>n</i> = 20)	10/2 ( <i>n</i> = 12)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.7 ± 4.2	24.9 ± 3.2	23.4 ± 3.3	25.2 ± 3.7
	Total cholesterol (mg/dL) <sup>a</sup>	191.9 ± 18.5	188.4 ± 23.9	177.4 ± 29.8 <sup>d</sup>	213.1 ± 22.1
	Hypertension, <i>n</i> (%)	4 (23.5%)	5 (31.3%)	4 (20%)	3 (25%)
	Diabetes, <i>n</i> (%)	3 (17.6%)	2 (12.5%)	5 (25%)	3 (25%)
	Smoking, <i>n</i> (%)	9 (52.9%)	7 (43.8%)	13 (65%)	8 (66.7%)
	Family history of CAD, <i>n</i> (%)	2 (11.7%)	2 (12.5%)	4 (20%)	3 (25%)
All cases	Sex M/F (total)	65/10 ( <i>n</i> = 75)	69/8 ( <i>n</i> = 77)	79/12 ( <i>n</i> = 91)	62/9 ( <i>n</i> = 76)
	Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	24.6 ± 3.9	24.5 ± 3.5	24.2 ± 3.7	25.3 ± 3.9
	Total cholesterol (mg/dL) <sup>a</sup>	186.4 ± 25.6	183.4 ± 23.1 <sup>c</sup>	170.6 ± 25.1 <sup>d</sup>	201.1 ± 21.2
	Hypertension, <i>n</i> (%)	8 (10.7%)	9 (11.9%)	11 (12.1%)	8 (10.5%)
	Diabetes, <i>n</i> (%)	8 (10.7%)	3 (3.9%)	8 (8.8%)	5 (65.8%)
	Smoking, <i>n</i> (%)	34 (45.3%)	41 (53.2%)	41 (45.1%)	36 (46.4%)
	Family history of CAD, <i>n</i> (%)	14 (18.7%)	14 (18.2%)	13 (14.3%)	16 (21.5%)

BMI, body mass index; CAD, coronary artery disease.

<sup>a</sup> Values are mean ± S.D.

<sup>b</sup> *P* < 0.05.

<sup>c</sup> *P* < 0.01.

<sup>d</sup> *P* < 0.001.

# Fatty streaks, fibrotic plaques and complicated lesions

**Table 2**

Mean area<sup>f</sup> of aortic atherosclerotic lesions in different age groups of patients with chronic *Opisthorchis felineus* infection.

Age (years)	AA lesion	Mild <i>O. felineus</i> burden <sup>a</sup>	Moderate <i>O. felineus</i> burden <sup>a</sup>	Severe <i>O. felineus</i> burden <sup>a</sup>	Non-infected controls <sup>a</sup>
20–29	Total <sup>b</sup>	4.6 ± 1.9	3.4 ± 1.3	2.6 ± 1.5 <sup>e</sup>	10.5 ± 5.6
	Fibrotic	0.2 ± 0.3	0.2 ± 0.5	0 <sup>e</sup>	0.3 ± 0.2
	Complicated	0	0	0	0
30–39	Total <sup>b</sup>	14.9 ± 6.7 <sup>d</sup>	9.2 ± 4.6 <sup>e</sup>	4.2 ± 3.4 <sup>e</sup>	22.1 ± 7.3
	Fibrotic	4.3 ± 5.5	3.9 ± 5.2	2.9 ± 1.8	5.2 ± 7.7
	Complicated	0.7 ± 0.5	0.7 ± 0.4 <sup>d</sup>	0.3 ± 0.4 <sup>d</sup>	0.7 ± 0.3
40–49	Total <sup>b</sup>	20.7 ± 7.6 <sup>c</sup>	13.4 ± 7.3 <sup>d</sup>	10.3 ± 5.9 <sup>e</sup>	34.1 ± 12.2
	Fibrotic	7.9 ± 7.4	6.2 ± 5.7 <sup>c</sup>	5.4 ± 3.9 <sup>c</sup>	11.3 ± 9.1
	Complicated	2.2 ± 2.1	1.7 ± 1.5 <sup>d</sup>	0.5 ± 0.7 <sup>e</sup>	3.7 ± 2.8
50–59	Total <sup>b</sup>	28.8 ± 9.7 <sup>c</sup>	21.4 ± 9.7 <sup>c</sup>	16.1 ± 6.2 <sup>e</sup>	43.7 ± 14.5
	Fibrotic	15.3 ± 9.2	13.1 ± 8.8 <sup>c</sup>	10.9 ± 8.7 <sup>c</sup>	18.2 ± 11.1
	Complicated	4.8 ± 5.1	3.7 ± 4.0 <sup>d</sup>	2.1 ± 1.5 <sup>d</sup>	6.1 ± 5.3
>60	Total <sup>b</sup>	40.4 ± 8.3	34.7 ± 8.5 <sup>e</sup>	22.5 ± 10.8 <sup>e</sup>	54.1 ± 18.1
	Fibrotic	17.1 ± 9.8	14.3 ± 8.1 <sup>c</sup>	12.2 ± 9.1 <sup>c</sup>	19.9 ± 11.2
	Complicated	5.3 ± 4.1	3.9 ± 3.4 <sup>c</sup>	3.3 ± 2.5 <sup>c</sup>	6.8 ± 6.5

<sup>a</sup> Values are mean ± S.D.

<sup>b</sup> Total atherosclerosis includes both fatty streak and fibrotic lesions.

<sup>c</sup>  $P < 0.05$ .

<sup>d</sup>  $P < 0.01$ .

<sup>e</sup>  $P < 0.001$ .

<sup>f</sup> Percentage: (single lesion area (mm<sup>2</sup>)/total area of the aorta (mm<sup>2</sup>)) × 100.

# *Opisthorchis felineus* CHI as a negative predictor of AA

**Table 3**  
Association of aortic atherosclerosis with *Opisthorchis felineus* chronic helminthic infection and worm burden.

Non-infected subjects (number)	<i>O. felineus</i> -infected subjects (number)	Univariate analyses OR (95% CI)	Multivariate analysis (adjusted for age and sex) OR (95% CI)
All subjects (76)	Total (243)	1.78 (1.06–3.01) <sup>a</sup>	1.72 (1.02–2.91) <sup>a</sup>
	Mild <i>O. felineus</i> burden (75)	1.27 (0.66–2.42)	1.14 (0.59–2.18)
	Moderate <i>O. felineus</i> burden (77)	1.74 (0.92–3.31)	1.57 (0.83–2.98)
	Severe <i>O. felineus</i> burden (91)	2.90 (1.52–5.56) <sup>c</sup>	2.34 (1.23–4.44) <sup>b</sup>
Subjects >40 years old (41)	Subjects >40 years old (148)	3.72 (1.57–8.82) <sup>b</sup>	3.19 (1.35–7.58) <sup>b</sup>
	Mild <i>O. felineus</i> burden (49)	2.58 (0.95–7.04)	1.94 (0.69–5.40)
	Moderate <i>O. felineus</i> burden (42)	4.42 (1.61–12.17) <sup>b</sup>	3.31 (1.19–9.16) <sup>a</sup>
	Severe <i>O. felineus</i> burden (57)	8.33 (3.14–22.08) <sup>c</sup>	6.22 (2.36–16.35) <sup>c</sup>

OR, odds ratio; CI, confidence interval.

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $P < 0.01$ .

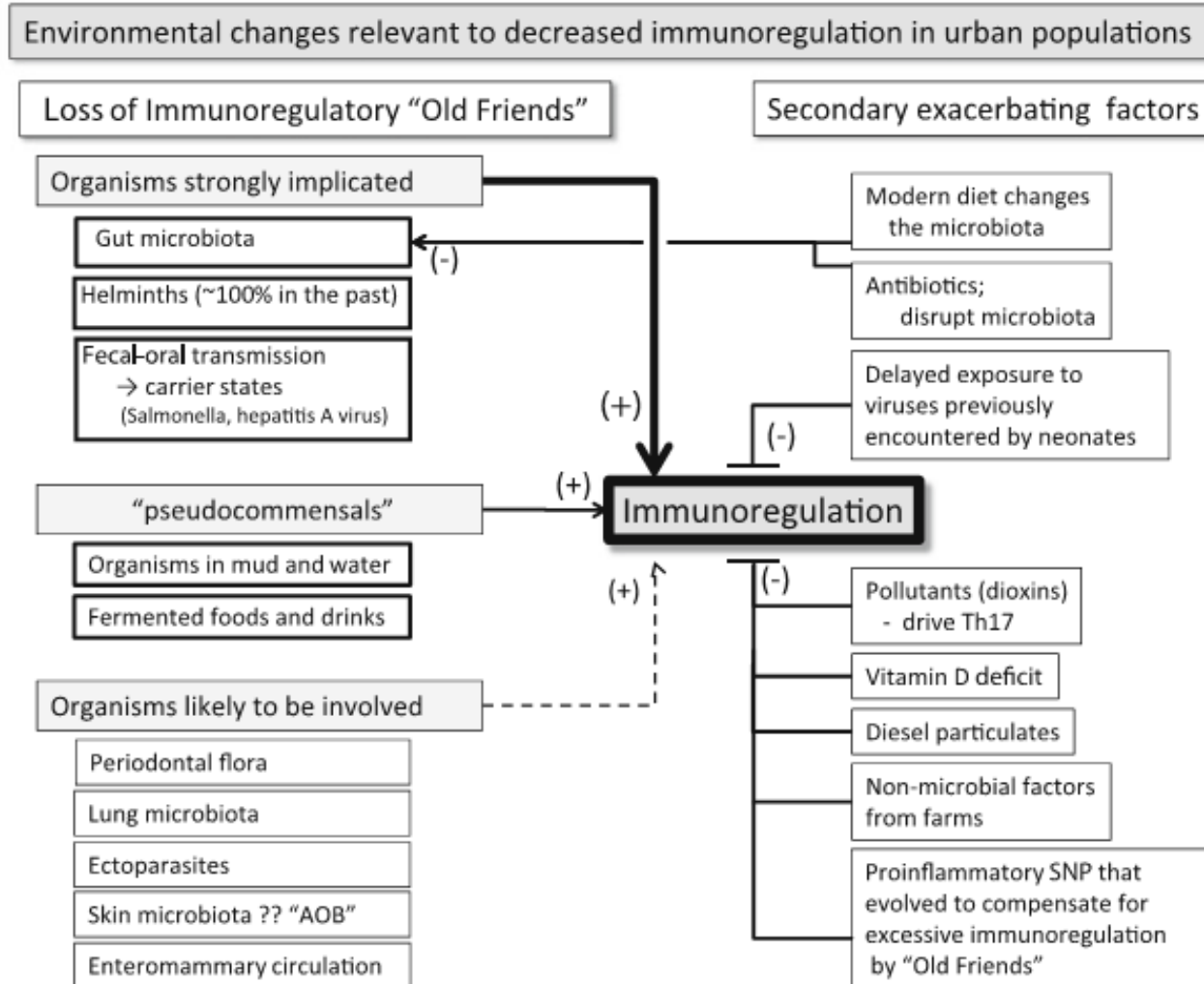
<sup>c</sup>  $P < 0.001$ .

# Conclusions

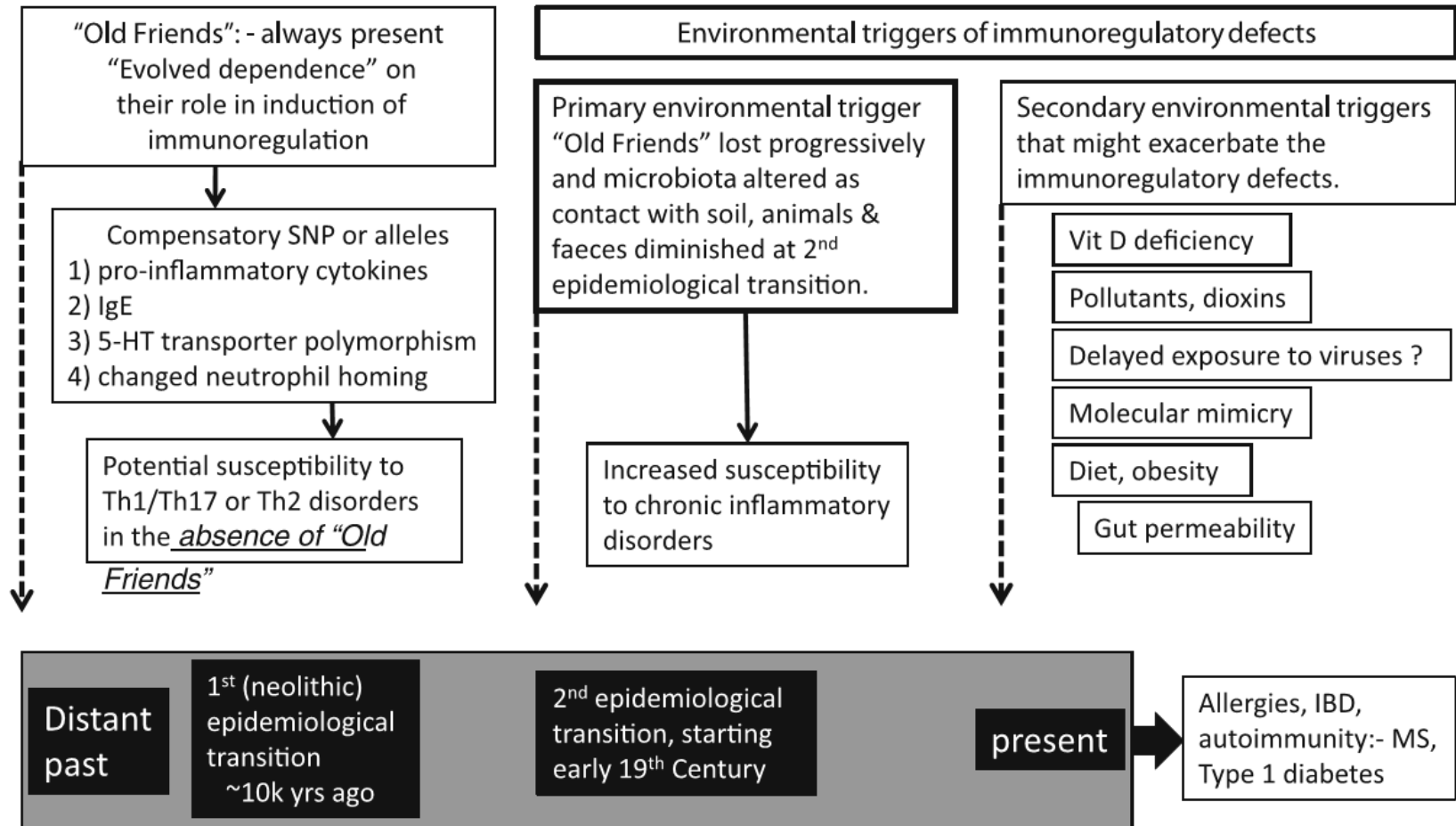
1. *O. felineus* CHI is associated with a reduction of serum total cholesterol levels, but without statistically significant correlation between serum total cholesterol levels and any type of atherosclerotic lesions in the study groups.
2. AA area in subjects with *O. felineus* CHI is lower than in uninfected gender- and age-matched controls;
3. *O. felineus* burden is the independent negative predictor of AA



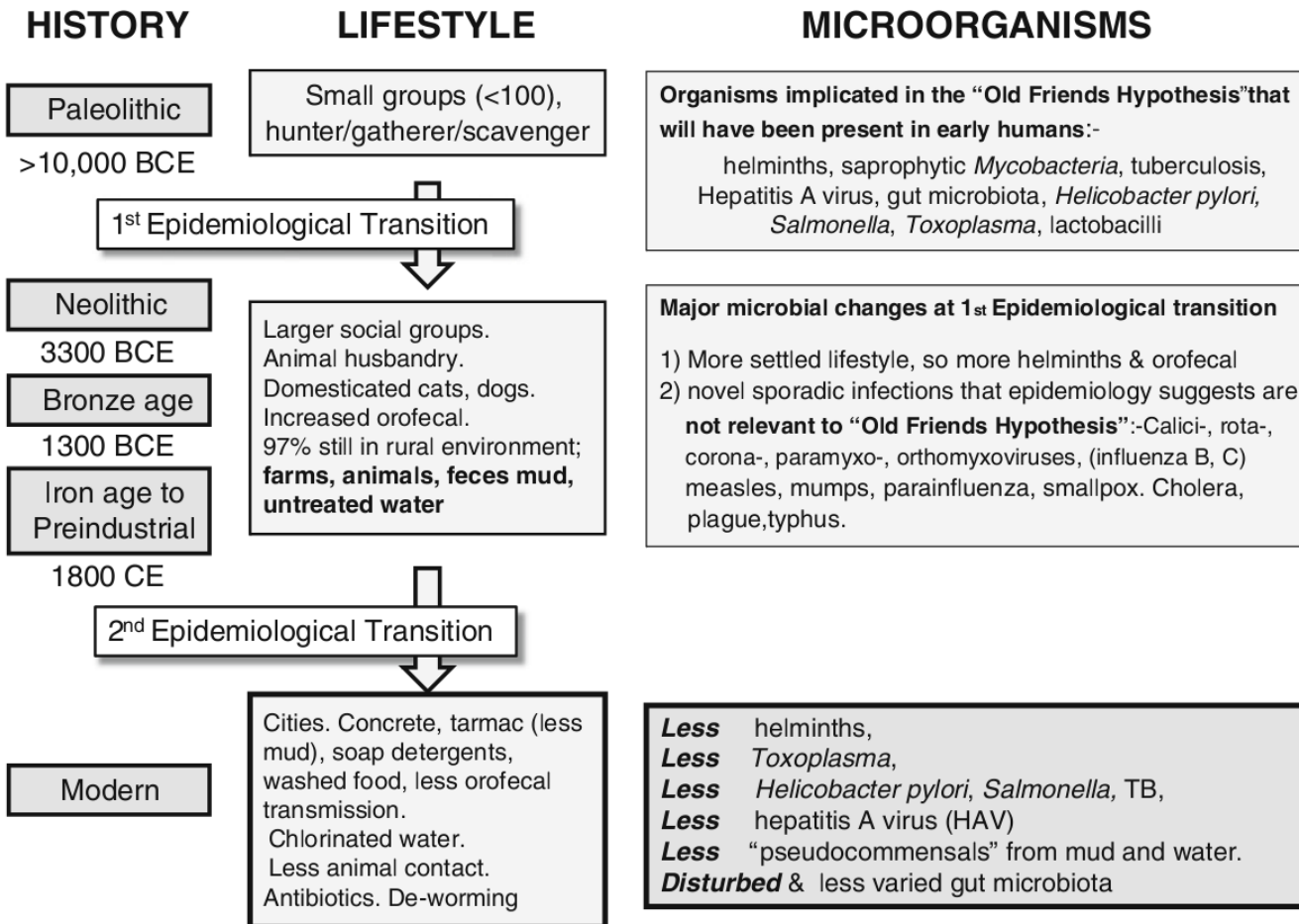
# Hygiene, or "Old Friends" Hypothesis



# Hygiene, or "Old Friends" Hypothesis



# Hygiene, or "Old Friends" Hypothesis



# Possible mechanisms of *O. felineus* anti-atherosclerotic action

- CHI is associated with a decrease in the synthetic ability of the liver, including its production of cholesterol (Doenhoff et al., 2002; Magen et al., 2005).
- Helminths are able to remodel/metabolise host lipids for their growth and to generate phospholipid membranes (Bansal et al., 2005).

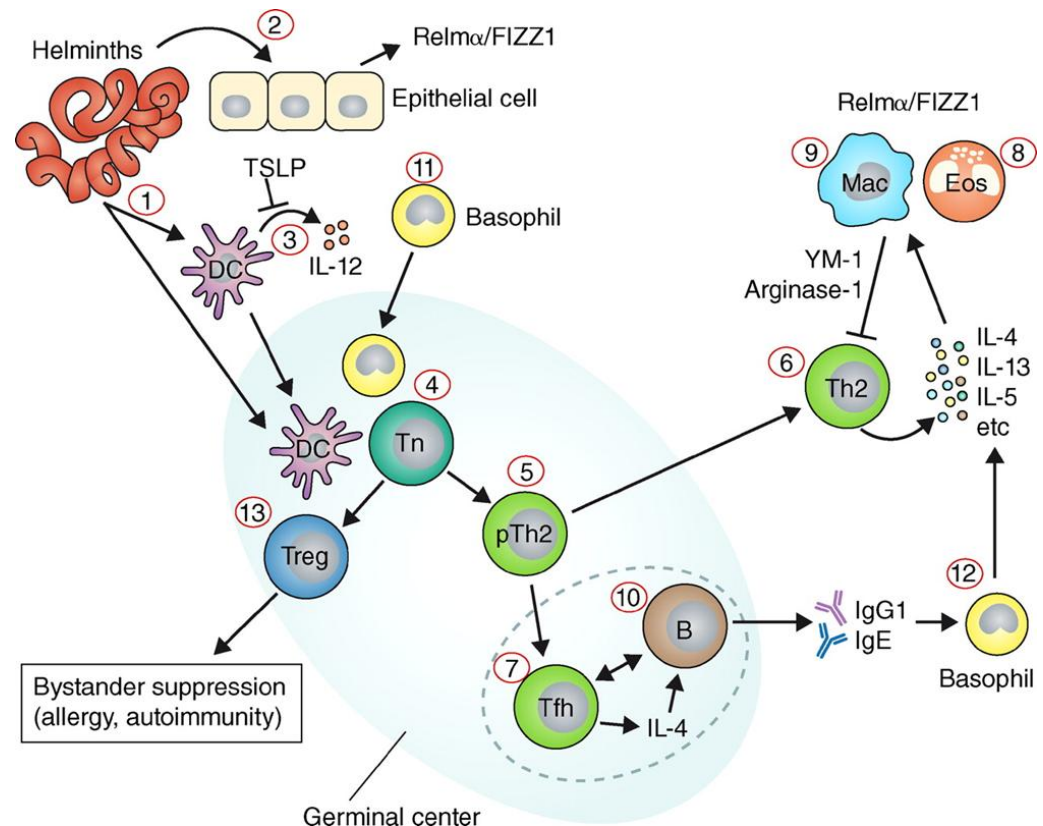
# Possible mechanisms of *O. felineus* anti-atherosclerotic action

- Helminth-induced immune modulation causing Th2 polarization can change lipid metabolism;
  - (total plasma cholesterol levels were found to be increased in Th1 polarised, IL-4-deficient or STAT-6-deficient mice (Huber et al., 2001; King et al., 2002)).

# Possible mechanisms of *O. felineus* anti-atherosclerotic action

By establishing chronic, mostly asymptomatic infection, helminths modulate dendritic cell function and induce strong immunomodulation that may include regulatory T cells, regulatory B cells and alternatively-activated macrophages

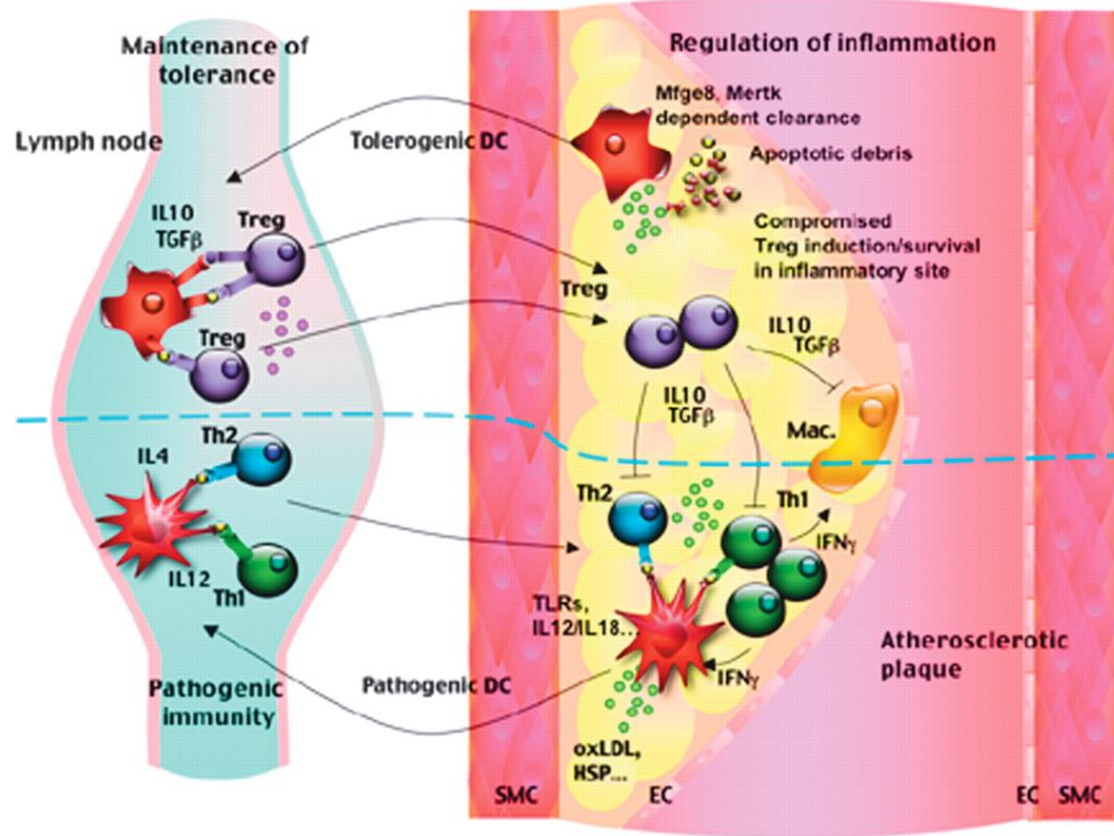
Fillatreau et al., 2008; Rausch et al., 2008; Carvalho et al., 2009; Gordon and Martinez, 2010; Allen and Maizels, 2011; Aranzamendi et al., 2013).



# Possible mechanisms of *O. felineus* anti-atherosclerotic action

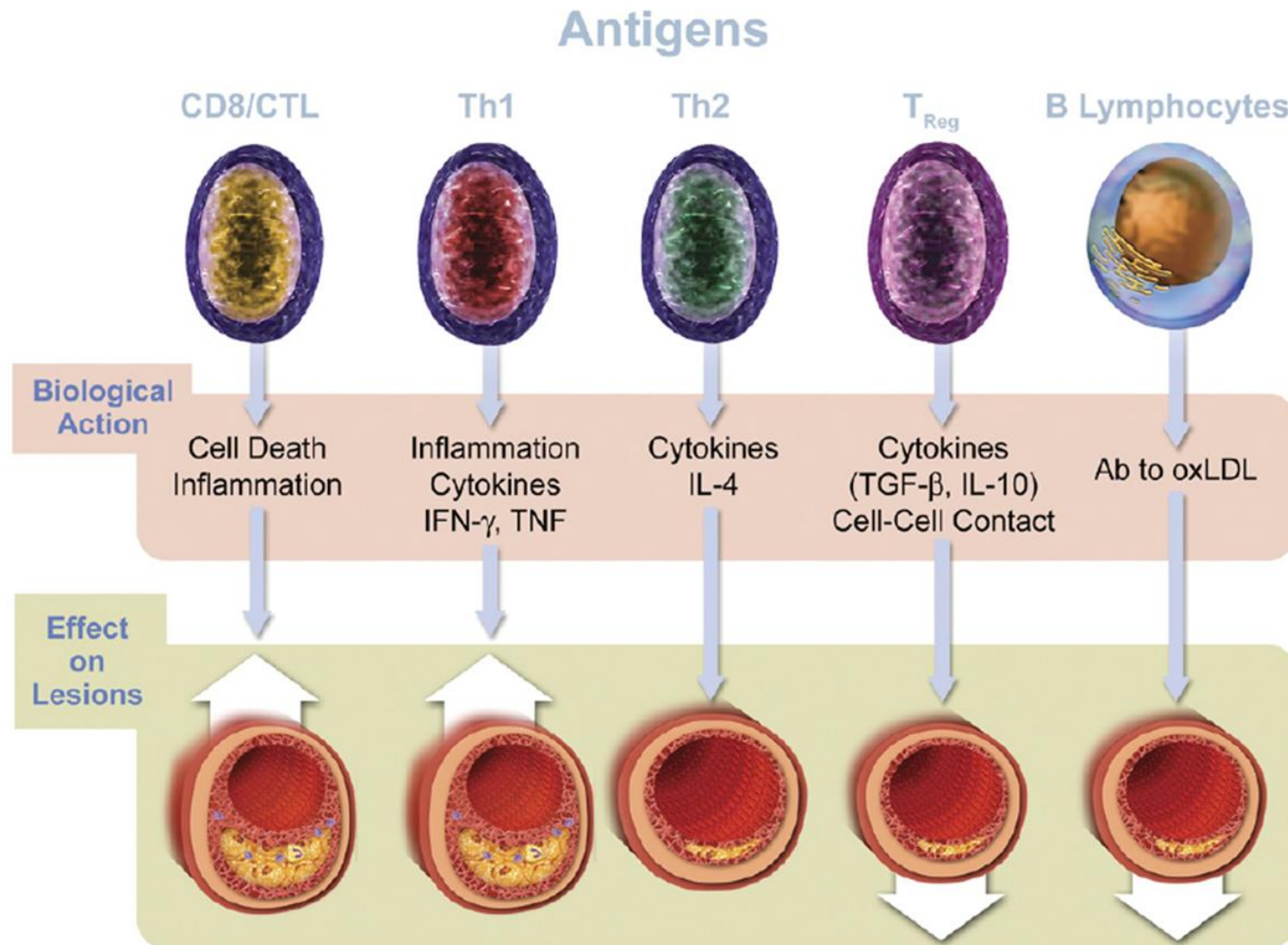
Atherosclerosis shares many similarities with other chronic autoimmune diseases.

The best-studied inflammatory cells in atherosclerotic lesions include T-cells, B-cells, dendritic cells and macrophages, while oxidised LDL, heat shock proteins and  $\beta$ 2-glycoprotein I are the most studied autoantigens.



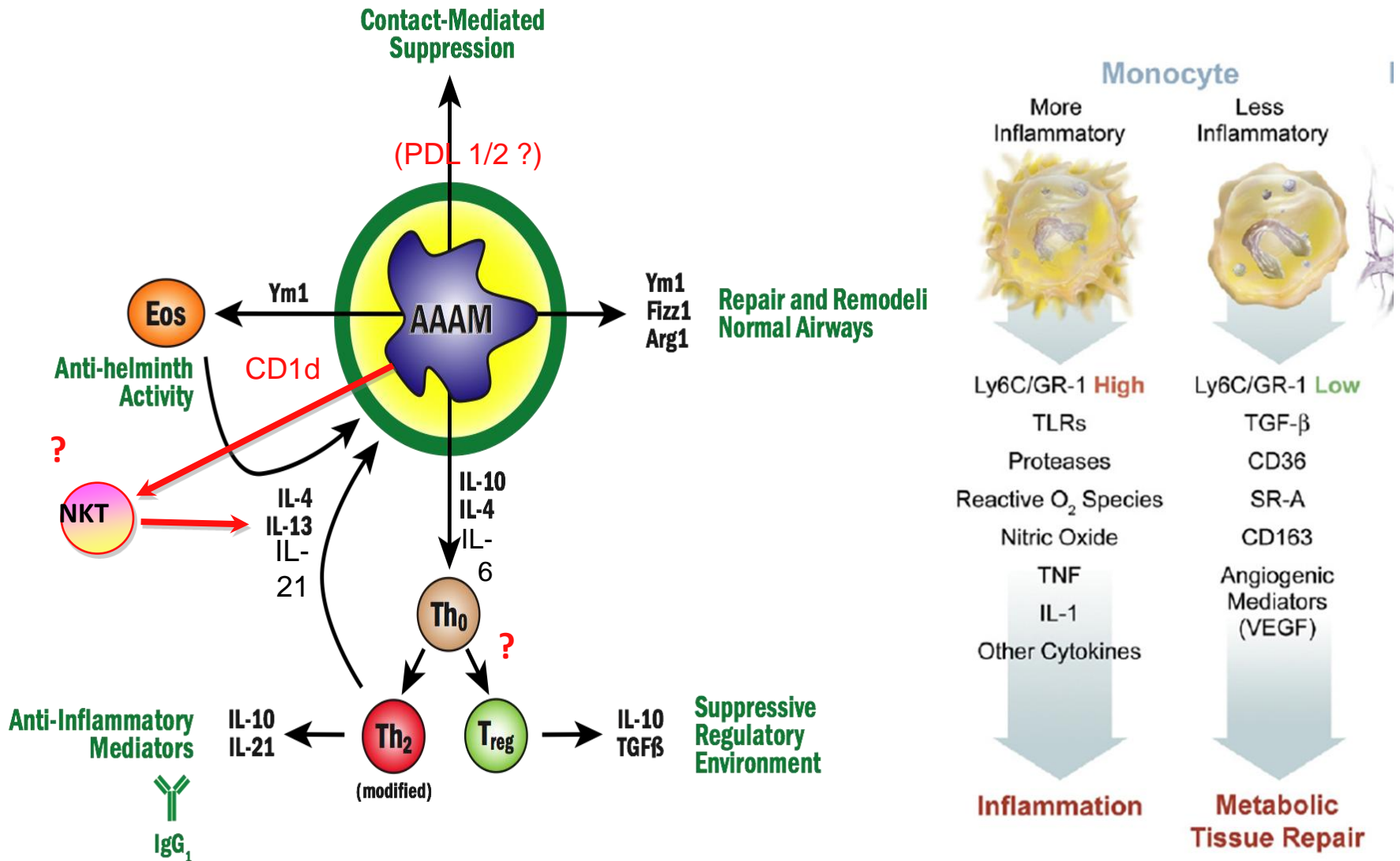


# Possible mechanisms of *O. felineus* anti-atherosclerotic action





# Alternatively Activated Macrophages



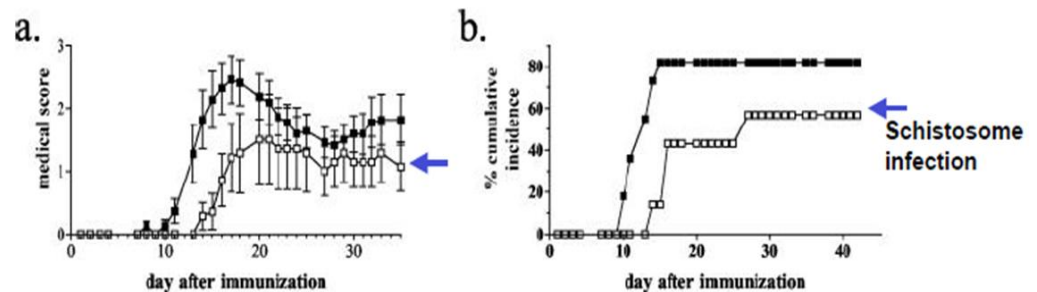
# Possible mechanisms of *O. felineus* anti-atherosclerotic action

To survive in the host, helminths have exerted significant selective pressure on mutations in genes implicated in immune function, modulating human susceptibility to several autoimmune diseases (Fumagalli et al., 2009).

At this time, it is not clear whether this mechanism is applicable to atherosclerosis.

In several models of autoimmune diseases  
helminth infections inhibit disease onset

Experimental allergic encephalomyelitis  
La Flamme et al 2003, Sewell et al 2003



Grave's thyroiditis  
Nagayama et al 2004

Collagen induced arthritis  
Mattsson et al 2000, McInnes et al 2003

Type -1 diabetes  
Cooke et al 1999, El-Waki et al 2002, Zacccone et al 2003,  
Saunders et al 2007

# Can worms defend our hearts?

- Multiple lines of evidence support the notion that there is an inverse relationship between helminthic infections and atherosclerosis and its related diseases.
- We hypothesized that worms are 'protective' against heart disease.
- Studies are needed to clarify the hypothesis.

**Can we use helminthic therapy (or  
helminth/antigen vaccination  
strategies) to protect against  
atherosclerosis?**