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Abstract

Advanced age is often associated with a chronic inflammatory status and inflammatory diseases. It has been suggested that exposure to infectious agents that stimulate the inflammatory response at early ages might have carry over effects in terms of accelerated senescence and increased mortality at late ages. However, not all pathogens and parasites have pro-inflammatory effects. In particular, parasitic nematodes have been shown to dampen the inflammatory response and to prevent or alleviate the symptoms of inflammatory diseases. We, therefore, tentatively predicted that early infection with a parasite that has anti-inflammatory properties might postpone aging. We tested this idea using the association between the nematode *Heligmosomoides polygyrus* and its rodent host. In addition to the infection with *H. polygyrus*, we also activated the systemic inflammatory response with an *Escherichia coli* LPS injection, to explore the effect of *H. polygyrus* under control and inflammatory conditions. In addition to lifespan, we also assessed several biomarkers of aging, once the infection had been cleared. We found that both treatments (*H. polygyrus* infection and LPS challenge) reduced longevity. Most of the biomarkers of aging were affected by the previous infection status, suggesting that mice exposed to the nematode had an accentuated senescent phenotype. These results show that infection with immunomodulatory parasites *per se* does not prolong host lifespan and rather support the view that infection in early life accelerates the rate of aging.

Keywords: Aging, *Heligmosomoides polygyrus*, Inflammation, LPS, Nematode

Highlights

- Infection with immunomodulatory parasites protects hosts against inflammatory diseases
- Aging is often associated with low grade chronic inflammation
- We predicted that infection at early ages with immunomodulatory parasites might retard the onset of aging
- Contrary to the prediction, mice infected at young ages with an immunomodulatory nematode had reduced longevity

1. Introduction

It is now well established that several degenerative, age-associated diseases have an inflammatory origin (Pawalec et al. 2014; Goldberg & Dixit 2015). Elderly are often characterized by a chronic, low grade inflammatory status that makes them more vulnerable to a range of pathologies including cardiovascular diseases and cancer (Franceschi et al. 2007; Vasto et al. 2007). While chronic inflammation can be harmful, the inflammatory response is with no doubt an essential component of the immunological arsenal against infectious diseases (Medzhitov 2008). With this respect, the inflammatory response might be seen as a function with antagonistic pleiotropic properties, conferring benefits in terms of protection towards pathogens, but also incurring costs in terms of induction of inflammatory diseases. Physiological functions with antagonistic pleiotropic properties might explain the evolution of aging, as suggested by George Williams (1957). Since the publication of this seminal paper, the antagonistic pleiotropy hypothesis for the evolution of aging has attracted considerable attention (Gaillard & Lemaitre 2017); however, the vast majority of studies focused on one of the predictions of the hypothesis, namely that there should be a negative correlation between early and late expressed traits (e.g., early and late fecundity, early growth and late mortality, etc.) (Lemaitre et al. 2015). The underlying physiological functions accounting for these age-dependent trade-offs have been much less studied. Recently, a couple of studies conducted on the mealworm (*Tenebrio molitor*) showed that an early immune challenge reduced individual lifespan and accelerated aging, while suppressing phenoloxidase (one of the immune effectors in insects) with RNAi partially restored longevity (Pursall & Rolff 2011; Khan et al. 2017). These results strongly point towards immunopathology as a possible mechanism of senescence in this model. Interestingly, these results are also in agreement with previous findings suggesting that early exposure to infectious diseases might account for old age mortality in human cohorts (Finch and Crimmins 2004).

Not all pathogens and parasites have similar effects on hosts and some of them actually stimulate immune regulatory effectors with anti-inflammatory properties (Johnston et al. 2009). The immune system imposes strong selection pressures on micro- and macro-parasites by reducing the infectious period (clearing the infection), or reducing parasite multiplication. In response to immune-mediated selection, parasites have evolved an astonishing diversity of immune evasion strategies (Schmid-Hempel 2008), including immune suppression mechanisms. Helminths are among the masters of immune regulation of their hosts (Maizels et al. 2004; Maizels & McSorley 2016). These macro-parasites profoundly rely on their capacity to dampen the host immune response to persist and establish chronic infections and, in many helminth species, the molecular/biochemical bases of such immunomodulatory strategies have been identified (McSorley et al. 2013). While being clearly beneficial to the parasite in terms of infection success, immunomodulation can also incur benefits to the host in terms of protection towards immune mediated diseases (Hang et al. 2013). Epidemiological and experimental evidence has shown that infection with helminths does confer a protection towards a range of inflammatory and immune diseases (Finlay et al. 2014). Such evidence is among the basis on which the hygiene hypothesis (Rook 2009), which aims at explaining the current epidemics of immune-mediated diseases (Bach 2002; Sorci et al. 2016), is built upon.

Given that the activation of the inflammatory response has been shown to accelerate the rate of aging and to reduce host longevity, it is straightforward to predict that parasites that exert no spoliation costs and protect from inflammatory disorders might actually retard host aging and promote host longevity. Here, we tested this prediction using the gastrointestinal nematode *Heligmosomoides polygyrus* and its rodent host (*Mus musculus domesticus*). *Heligmosomoides polygyrus* is a natural parasite of mice that has become a model organism in immunoparasitology (Maizels et al. 2012). *Heligmosomoides polygyrus* secretes a diversity of proteins with immunomodulatory properties that stimulate regulatory T cells, and interfere with

antigen presenting cells (McSorley et al. 2013). *Heligmosomoides polygyrus* infected mice have a dampened immune response and are protected against the symptoms of experimentally induced inflammatory diseases (Setiawan et al. 2007; Sutton et al. 2008).

We infected a susceptible strain of mice (CBA) with *H. polygyrus* when they were seven weeks old. In addition to the infection, mice were also exposed to a systemic inflammatory challenge, at the age of 11 weeks, with an *Escherichia coli* LPS injection. This allowed exploring the effect of *H. polygyrus* under control and inflammatory conditions. Mice were then paired to assess reproductive success and subsequently monitored for their survival until the entire cohort extinguished. We also assessed different biomarkers of aging. We predicted that *H. polygyrus* infected individuals should have delayed aging and prolonged lifespan compared to non infected mice. LPS injected individuals are predicted to have the shortest lifespan and *H. polygyrus* infected mice that were also LPS challenged are predicted to have lifespan in between LPS injected and *H. polygyrus* infected mice.

2. Methods

2.1 Experimental procedure

Female CBA mice (n = 120) were purchased from Janvier Labs (Laval, France) and housed in plastic cages (18.5 cm x 38 cm x 22.5 cm), five individuals per cage. Mice were kept under standardized conditions (temperature: 21°C; light/dark cycle: 12 h/12 h) at the mouse house of the Université de Bourgogne and provided with commercial mouse pellets and filtered tap water *ad libitum*. When seven weeks old, half of the mice (n = 60) were infected with 300 L3 larvae of the gastrointestinal nematode *Heligmosomoides polygyrus* suspended in 200 µl of water. Mice were infected by oral gavage. The other sixty mice were given the same volume of water with no *H. polygyrus* larvae. When mice were 11 weeks old, half of them (30 infected with *H. polygyrus* and 30 non infected) were injected intraperitoneally with LPS from *Escherichia coli* (serotype 055: B5; Sigma, St. Louis; 1 µg g^{-1}) in 100 µl of phosphate buffered

saline (PBS). The other half received the same volume of PBS. Twenty-four hours post-LPS injection, each female was placed in a cage with a randomly selected male CBA mouse for reproduction. After 10 days, males were removed and females were first left alone and then kept with their pups until weaning. We monitored litter size at birth and at weaning (when pups were 21 days old). Female mice were then replaced in their shared cages (5 per cage) and their survival monitored until the entire cohort extinguished. Senescing mice that reached the end point, defined as a rapid weight loss (20% body mass), abnormal physical appearance and behavior (prostration, inability to ambulate), or presence of clinical signs (respiratory distress, tumors) were humanely euthanized. All animal experiments were approved by the Comité d'Ethique de l'Expérimentation Animale Grand Campus Dijon, France (CNREEA n° C2EA – 105) (#1212, June 10th 2012).

2.2 Biomarkers of aging

We assessed several biomarkers of aging when mice were between 17 and 26 months old (i.e., after parasites had been expelled in all mice). We assessed: i) red blood cell and platelet counts (n = 55 mice), ii) eosinophilia and neutrophil-to-lymphocyte ratio (n = 55 mice), iii) blood glycaemia (n = 41 mice), iv) leukocyte telomere length (n = 34), v) the composition of gut microbiota (n = 24 mice). We also measured body mass of all surviving mice when they were 16, 18, 21, and 27 months old.

When mice were 17 months old, we collected 10 µl of blood from the tip of the tail in the morning (10:00 am). Blood cells (lymphocytes, neutrophils, eosinophils, red blood cells and platelets) were counted using a Scil Vet abc+ haematology analyser.

When mice were 19 months old, we measured glycaemia. A drop of blood was collected from the tip of the tail in the morning (10:00 am) and blood glucose (mg/dl) measured using an Accu-Chek Performa device (Roche GmbH Diagnostic).

Leukocyte telomere length was assessed using a real-time quantitative PCR (Rt-qPCR) (Cawthon 2002) derived from (Grosbellet et al. 2015). We collected a blood sample, by submandibular vein puncture, when mice were 24 months old. Blood was rapidly centrifuged (4000 rpm, 4° C, 10 min), plasma was removed and packed blood cells were immediately stored at -80°C. Detailed information on the methods used to assess telomere length is provided in the online supporting information.

The composition of the gut (fecal) microbiota of 26 months old mice was assessed using a metabarcoding technique based on the 16S bacterial gene. Fresh feces were collected, immediately frozen and stored at -80° C. Detailed information on the methods used to assess the diversity and composition of the gut microbiota is provided in the online supporting information.

2.3 H. polygyrus *infection monitoring*

The persistence of *H. polygyrus* infection was assessed monitoring the presence of parasite eggs in mouse feces. We checked the persistence of the infection at 3, 7 and 16 months post-infection. To this purpose, mice were placed in clean individual cages from 8 to 12 a.m. Thereafter, mice were placed back into their home cage, and the feces were collected. Feces were weighed and suspended in a 50% NaCl solution. The suspension was then placed in a McMaster counting chamber for 15 min for flotation. Egg number was counted in duplicate (intraclass correlation coefficient R = 0.99, n = 34), and the mean values used in the statistical analyses.

2.4 Statistical analyses

Lifespan was analysed using a two-way ANOVA with the two treatments and their interaction. To take into account the possible confounding effect of reproductive effort, we also ran an ANCOVA model that included either litter size, either the number of young at weaning as covariates. To explore time-dependent mortality, we ran a Cox regression model that

included the two treatments and their interaction. Blood cell counts, glycaemia, and telomere length were analyzed using two-way ANOVAs with the two treatments and their interaction. Alpha and beta diversity of the microbiota were computed using QIIME software v. 1.9.1. We checked the correlation of Shannon diversity index between duplicated PCR samples (Pearson R = 0.88) and merged the sequences. To normalize the sequencing depth, we estimated alpha and beta diversity on 1,000 rarefied OTU tables to the minimal sequencing depth (12,000 sequences) and considered the mean values for each metric. We estimated the microbiota diversity within mice using Shannon index (alpha diversity) and the dissimilarity of the composition of microbiota between mice (beta diversity) using the following metrics: Bray-Curtis, Jaccard, unweighted and weighted Unifrac. We ran a two-way ANOVA with alpha diversity as the dependent variable and the two treatments and their interaction as independent variables. We characterized the variation of beta diversity using a principal coordinate analysis for each dissimilarity index. We used PERMANOVA (function Adonis through Vegan package, 10,000 permutations) to test the effect of the two treatments (*H. polygyrus* infection and LPS challenge) and their interaction on the dissimilarity of the microbiota composition. We also explored whether the abundance of bacterial families differed according to the treatments. This was done using non parametric *t* tests (10,000 permutations) implemented in QIIME v. 1.9.1, based on averaged abundances of 1,000 rarefied tables.

Changes in body mass were analyzed using a general linear mixed model to take into account the repeated nature of the data. The model included age, squared age, age at death (to correct for selective disappearance), the two treatments and the interactions as fixed factors, and mouse identity as a random factor. Degrees of freedom were approximated using the Satterthwaite method. Analyses were done using R (3.3) and SAS (9.2).

3. Results

3.1 H. polygyrus *infection monitoring*

We checked the persistence of *H. polygyrus* at month 3, 7 and 16 post-infection. All infected mice shed parasite eggs 3 months post-infection (mean ± se = 44,984 ± 5,450 eggs g^{-1} of feces, n = 46), although PBS treated mice shed more eggs than LPS challenged individuals (see Guivier et al. 2016). At 7 months post-infection, the number of eggs shed in the feces of infected mice had dropped by 95% (mean ± se = 2,323 ± 385 eggs g^{-1} of feces, n = 46), and 13 individuals out of 46 had cleared the infection. By month 16 post-infection, all mice had cleared the infection.

3.2 Longevity

Lifespan was analyzed with a two-way ANOVA with *H. polygyrus* infection and LPS challenge as factors. The analysis showed a strong effect of the interaction between *H. polygyrus* infection and LPS challenge (*H. polygyrus* infection, $F_{1,116} = 25.13$, $p < 0.0001$; LPS challenge, $F_{1,116} = 33.23$, $p < 0.0001$; *H. polygyrus* infection x LPS challenge, $F_{1,116} = 12.48$, $p = 0.0006$), with *H. polygyus* infected and LPS injected mice having the shortest lifespan (fig. 1A).

The LPS injection in *H. polygyrus* infected mice produced a rapid mortality in the 48 hours that followed the injection (see Guivier et al. 2016), which was obviously not due to aging. We therefore also analyzed the data restricting them to the individuals who survived this early episode of mortality (n = 106). This model showed that *H. polygyrus* infection x LPS challenge interaction did not predict lifespan anymore ($F_{1,102} = 0.42$, $p = 0.5204$; fig. 1B). However, *H. polygyrus* infection and LPS challenge had an additive effect on lifespan. *H. polygyrus* infected mice had a median longevity of 794 days vs. 871 days for non infected mice ($F_{1,103} = 6.61$, $p = 0.0116$; fig. 1B), and LPS injected mice had a median longevity of 791 days vs. 869 days for PBS injected mice ($F_{1,103} = 7.77$, $p = 0.0063$; fig. 1B).

The additive effect of the two treatments did not depend on the reproductive effort produced when mice were 12 weeks old. Neither the number of young at birth nor the number of young at weaning explained lifespan ($F_{1,102} = 0.82$, $p = 0.3684$ and $F_{1,102} = 1.00$, $p = 0.3192$, respectively) whereas the effect of *H. polygyrus* infection and LPS challenge remained significant whatever the measure of reproductive effort that was included in the model (number of young at birth: *H. polygyrus* infection, $F_{1,102} = 6.49$, $p = 0.0123$, LPS challenge, $F_{1,102} = 7.02$, $p = 0.0093$; number of young at weaning: *H. polygyrus* infection, $F_{1,102} = 6.34$, $p = 0.0134$, LPS challenge, $F_{1,102} = 6.73$, $p = 0.0109$). None of the interactions was statistically significant (all p's > 0.4).

We also used a Cox regression model to analyze time-dependent mortality. This model confirmed that when using the whole dataset, there was a statistically significant interaction between *H. polygyrus* infection and LPS challenge ($\chi^2_1 = 5.08$, $p = 0.0242$). When the individuals that died immediately following the LPS injection were censored, the interaction was no longer significant and the infection status and the LPS challenge had additive effects on age-dependent survival probability (table 1; fig. 1C).

3.3 Biomarkers of aging

We explored the effect of the two treatments (*H. polygyrus* infection and LPS injection) on red blood cell and platelet counts, and on eosinophilia and neutrophil-to-lymphocyte ratio when mice were 17 months old. Blood was collected once the infection had been cleared and therefore any difference between groups reflects long-term, persistent effects of the two treatments.

Red blood cell and platelet counts were affected by the past infection status and, for both variables, *H. polygyrus* infected mice had lower values (red blood cells, *H. polygyrus* infection: $F_{1,51} = 5.69$, $p = 0.0208$; LPS challenge, $F_{1,51} = 0.22$, $p = 0.6404$; *H. polygyrus* infection x LPS challenge, $F_{1,51} = 2.78$, $p = 0.1015$; platelets, *H. polygyrus* infection: $F_{1,51} = 8.53$, $p = 0.0052$;

LPS challenge, $F_{1,51} = 0.00$, $p = 0.9620$; *H. polygyrus* infection x LPS challenge, $F_{1,51} = 0.74$, $p = 0.3924$; fig. 2A,B).

Mice previously infected with *H. polygyrus* maintained higher eosinophil numbers (expressed as percent of total white blood cells) compared to non infected mice (*H. polygyrus* infection: $F_{1,51} = 8.65$, $p = 0.0049$; LPS challenge, $F_{1,51} = 0.11$, $p = 0.7381$; *H. polygyrus* infection x LPS challenge, $F_{1,51} = 0.13$, $p = 0.7194$; fig. 3). The neutrophil-to-lymphocyte ratio was not affected by the *H. polygyrus* infection nor by the LPS challenge (*H. polygyrus* infection: $F_{1,51} = 0.78$, $p = 0.3816$; LPS challenge, $F_{1,51} = 0.53$, $p = 0.4679$; *H. polygyrus* infection x LPS challenge, $F_{1,51} = 0.40$, $p = 0.5323$).

We measured glycemia in the blood when mice were 19 months old. We did not find any effect of the two treatments on blood glycaemia (*H. polygyrus* infection, $F_{1,37} = 0.02$, $p = 0.9028$; LPS challenge, $F_{1,37} = 1.26$, $p = 0.2695$; *H. polygyrus* infection x LPS challenge, $F_{1,37} = 1.08$, $p = 0.3057$).

Telomere length was assessed when mice were 24 months old. We found that telomere length was affected by the infection status, *H. polygyrus* infected mice having longer telomeres ($F_{1,31} = 37.45$, $p < 0.0001$; fig. 4). Neither the LPS injection ($F_{1,31} = 0.64$, $p = 0.4289$), nor the interaction between *H. polygyrus* infection and LPS challenge affected telomere length ($F_{1,30} = 0.23$, $p = 0.6381$).

The composition of the gut microbiota has been shown to shape health in the elderly (Claesson et al. 2012). We therefore investigated if the two treatments affected the composition of the gut microbiota when mice were 26 months old. We obtained a total of 568,071 denoised reads, with 389, 382, 393 and 364 unique OTUs in *H. polygyrus* infected, non infected, LPS and PBS mice, respectively. Alpha diversity (Shannon index) of the microbiota was lower in LPS challenged mice than in PBS mice ($F_{1,20} = 4.55$, $p = 0.045$). Neither the infection with *H. polygyrus* nor the interaction between the two treatments had an effect on alpha diversity (*H.*

polygyrus infection, $F_{1,20} = 1.48$, $p = 0.24$; *H. polygyrus* infection x LPS challenge, $F_{1,20} = 0.28$, $p = 0.60$). The analysis of beta diversity showed that the composition of the microbiota differed between experimental groups for both treatments, when using the Jaccard dissimilarity index (table 2). When using the other metrics (Bray-Curtis, unweighted and weighted Unifrac) there was no difference in beta diversity between the experimental groups (table 2). Given that Jaccard index is based on the presence/absence of OTUs in different samples, we compared the differences in relative abundance of bacterial families between the groups (fig. 5A). *H. polygyrus* infected mice had a lower abundance of *Lachnospiraceae* compared to non infected individuals (non-parametric *t* test, $t = -4.06$, $p = 0.0003$, $p = 0.036$ after FDR correction, fig. 5B).

Finally, we measured body mass when mice were 16, 18, 21 and 27 months old. Body mass was relatively stable up to the age of 21 months and then dropped at the age of 27 months. Previously infected mice tended to have higher values of body mass, however age-dependent trajectories were similar for the different experimental groups, as shown by non-significant interactions between age (and squared age) and treatments (table 3, fig. S1).

4. Discussion

We suggested and tested the idea that asymptomatic infection with immunomodulatory parasites might retard the onset of senescence in hosts and improve their lifespan. This idea is rooted into a well-established body of evidence. First, many helminth parasites, and nematodes in particular, dampen the host immune and inflammatory response to ensure the establishment of chronic infections (Maizels & McSorley 2016). Second, aging is often associated with the up-regulation of low grade chronic inflammation. “Inflammaging” provides a favorable ground for the development of many age-associated syndromes including cancer and cardiovascular diseases (Vasto et al. 2007). Contrary to the prediction, we found that mice infected with the

gut nematode *Heligmosomoides polygyrus* had shortened lifespan. Well after having cleared the infection, mice that had been infected with the nematode also had accelerated senescence in red blood cell and platelet counts, maintained high eosinophilia, and harbored distinct gut microbiota. A notable exception to this general rule was provided by the results on telomere length, showing that mice that had been infected with *H. polygyrus* had longer telomeres.

Aging is defined as the progressive decline in organismal performance ultimately leading to accelerated age-dependent mortality and decreased age-dependent reproductive output (Kirkwood & Austad 2000). The study of aging requires exploring the molecular, biochemical and physiological mechanisms underpinning such age-associated decline in performance, as well as the forces underlying its evolution and persistence. The main evolutionary theories of aging share the same basic assumption, namely that the strength of natural selection declines with age (Hamilton 1966). As a consequence, deleterious mutations can accumulate during lifespan (Medawar 1952) and genes with deleterious effects at late ages can be positively selected if they provide benefits at early ages, when selection is strong (Williams 1957). This last hypothesis has been coined the antagonistic pleiotropy theory of aging (Williams 1957).

The mechanisms underlying the trade-off between early and late performance remain, however, poorly understood. Inflammation might be one of such functions with pleiotropic properties. The inflammatory response is an essential component of organismal homeostasis, protecting against infection and restoring tissue integrity after trauma (Medzhitov 2008). Nevertheless, chronic inflammation can also produce damage and trigger many diseases occurring at old ages (Okin & Medzhitov 2012). This set of observations makes the inflammatory response an ideal candidate for a function with antagonistic pleiotropic properties over time (van den Biggelar et al. 2004). Based on this knowledge, we therefore predicted that individuals protected from inflammatory insults in early life should enjoy retarded senescence

and improved longevity. In a way, this represents a complementary test of the hypothesis put forward by Finch and Crimmins (2004) who, based on age-dependent mortality of human cohorts in pre-industrial Sweden, suggested that early exposure to inflammatory threats (during childhood) correlates with increased adult mortality rate.

Nematodes have been shown to be masters of immune regulation (Maizels et al. 2004). While this is supposed to allow the parasite to establish long-lasting infection, there is now accumulating evidence that it can also benefit the host in terms of protection towards immune and inflammatory disorders, and short-term alleviation of inflammatory symptoms (Bashi et al. 2015). We therefore predicted that mice infected with the immunomodulatory nematode *Heligmosomoides polygyrus* at young age should have postponed senescence and longer lifespan. We actually found the opposite pattern and infected mice suffered from the highest mortality rate at old ages.

In addition to the age-dependent mortality, we also assessed a number of markers of aging and found that *H. polygyrus* infected mice had i) lower red blood cell and platelet counts, ii) higher eosinophilia, iii) longer leukocyte telomeres, iv) distinct gut microbiota composition. Decreased red blood cell and platelet count is a common feature of the senescent phenotype in humans and mice (Guralnik et al. 2004; Guo et al. 2014; Jones 2016). Age-related anemia and muscle loss are associated with increased physical disability (Penninx et al. 2004), whereas functional changes in platelets with age are less well known (Jones 2016). Mice that had been previously infected by *H. polygyrus* were more anemic than non infected mice at the same age and had lower platelet counts, corroborating the idea that individuals that were exposed to the infection suffered from an earlier onset of senescence.

Eosinophilia is an almost universal feature of helminth infection; however, the role played by eosinophils in the resistance to helminths is not fully elucidated (Grencis 2015). Whatever the function of eosinophilia, we found that mice maintained elevated number of

circulating eosinophils well after the parasite had been cleared. This shows that previous infection induced long-lasting changes in host immune functions.

The study of the gut microbiota has received a lot of attention in the last decade following the discovery of its pervasive effects on host physiology and homeostasis (Honda & Littman 2016; Schroeder & Backhed 2016). Dysbiosis has been associated with several pathologies (Cho & Blaser 2012), and recent work has shown that the diversity and composition of the microbiota change at old age in both humans and mice (Langille et al. 2014; O'Toole & Jeffery 2015). We did not monitor age-dependent changes in the microbiota; however, we showed that both treatments produced long lasting effects on the diversity and composition of the gut microbiota. These differences were visible well after *H. polygyrus* infection had been cleared showing a permanent alteration of the gut microbiota. In particular, we found that mice that had been exposed to the LPS challenge harbored a microbiota with reduced alpha diversity, while beta diversity differentiated mice belonging to the two treatments. Interestingly, abundance of *Lachnospiraceae* was reduced in mice that had been infected with *H. polygyrus*. Recent work has shown that while *Lachnospiraceae, Bacteroidaceae* and *Ruminococcaceae* are the most abundant families in the human gut microbiota, their cumulative relative abundance gradually declines with advancing age [from 77.8% in young (mean age 30.5 years) to 57.7 % in super centenarians (mean age 106.2 years)] (Biagi et al. 2016). The observed decrease in abundance in *Lachnospiraceae* in *H. polygyrus* infected mice is therefore in agreement with the hypothesis of accelerated aging in these individuals.

We found that *H. polygyrus* infected mice had longer telomeres compared to non infected individuals. Telomere attrition has often been reported to be a biomarker of aging (Muezzinler et al. 2013), accelerated by inflammation and oxidative stress (Zhang et al. 2016). Evidence on the causal relationship between telomere length and aging remains, however, scant (Simons 2016). We did not measure telomere attrition; however, since individuals were

randomly assigned to the different experimental groups, we can assume that telomere length at the age of 7 weeks (when the experiment started) was similar among treatments. The finding that infected mice had longer telomeres at an advanced age seems at odd with the other results reported here, and with previous finding on the effect of infection on telomeres (Ilmonen et al. 2008). It should be noted, however, that evidence on the link between infection and telomeres comes from studies on micro-parasites (e.g., van de Berg et al. 2010). To our knowledge, this is the first study investigating how infection with a gut nematode, with anti-inflammatory properties, affects telomere length. Further work is definitely needed to establish whether the anti-inflammatory effects of helminths might extend to a protective function towards telomere attrition.

The idea that immunomodulatory parasites might dampen senescence is based on the assumption that the benefits of immune modulation, in terms of protection towards inflammatory diseases, outweigh any potential costs induced by the exploitation of the host. Susceptible mouse strains generally tolerate *H. polygyrus* infection with no apparent (or minimal) fitness cost, unless the infective dose exceeds a threshold (Lippens et al. 2016). We used a tolerant mouse strain and infected it with a parasite dose that was not supposed to incur spoliation costs. In agreement with this, we found that *H. polygyrus* infected mice had similar survival rate to non infected mice during the infectious period and had similar reproductive output during peak infection (see Guivier et al. 2016). Therefore, we believe that the observed accelerated senescence and the reduced longevity of *H. polygyrus* infected mice does not result from a direct spoliation effect of the parasite. Contrary to CBA, other mouse strains such as SJL or BALB/c produce an effective Th2 immune response against *H. polygyrus* (Filbey et al. 2014) and as such clear the infection at a much faster rate than CBA mice. A very useful extension of the current work would, therefore, be to use a resistant mouse strain, to investigate

i) whether the observed results depend on the establishment of a long-lasting, chronic infection, and ii) the underlying immunological mechanisms.

The other assumption of our hypothesis is that *H. polygyrus* successfully dampen the host inflammatory response. Several studies have shown that *H. polygyrus* infection stimulates regulatory T cells that dampen both Th1 and Th2 responses (Setiawan et al. 2007; Hang et al. 2013). Ultimately, this often produces the alleviation of inflammatory symptoms, as in the case of chemically induced colitis in *H. polygyrus* infected mice (Sutton et al. 2008; Lippens et al. 2017). At specific stages of *H. polygyrus* life cycle, hosts can nevertheless be transitory exposed to inflammatory bursts. Following ingestion by the host, infective larvae penetrate into the intestinal wall where they undergo two molts. Adult worms emerge back into the intestinal lumen where they reproduce. Larvae penetration and adult emergence may lead to the leakage of bacterial cells from the intestinal lumen, providing a stimulus for the inflammatory response. In a companion paper, we assessed the production of pro- and anti-inflammatory cytokines at day 28 post-infection (Guivier et al. 2016). As expected, we found that *H. polygyrus* infected mice had an up-regulated production of the anti-inflammatory cytokine TGF-β; however, infected mice also produced higher amounts of the pro-inflammatory cytokine IFN-γ (Guivier et al. 2016). This up-regulation of the inflammatory response might, therefore, account for the increased mortality at old age reported here. Interestingly, an up-regulated IFN-γ response has also been reported in CBA as well as in other mouse strains that are susceptible to *H. polygyrus*, such as C57BL/6, both during single infections (Filbey et al. 2014) and during co-infections with malaria parasites (Helmby 2009). Therefore, as already mentioned, it would be very interesting to investigate the long-term effects of *H. polygyrus* on aging of mouse strains that have a canonical anti-helminthic response based on Th2 effectors.

To conclude, whatever the mechanisms underlying the accelerated aging of early infected individuals, we showed that infection with a gut nematode at young age produced faster

senescence and altered a series of biomarkers of aging. These findings suggest that parasites that do not produce immediate fitness costs (in terms of induced mortality or reproductive failure) can still have long lasting (once the infection has been cleared) negative consequences on host aging.

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References

Bach JF. 2002. Mechanisms of disease: The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 347: 911–920.

Bashi T, Bizzaro G, Ben-Ami Shor D, Blank M, Shoenfeld Y. 2015. The mechanisms behind helminth's immunomodulation in autoimmunity. Autoimmun Rev. 14: 98-104.

Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, Consolandi C, Quercia S, Scurti M, Monti D, Capri M, Brigidi P, Candela M. 2016. Gut microbiota and extreme longevity. Curr Biol. 26: 1480-1485.

Cawthon RM. 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30: e47.

Cho I, Blaser MJ. 2012. The human microbiome: at the interface of health and disease. Nature Rev Genet. 13: 260–270.

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HMB, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. 2012. Gut microbiota composition correlates with diet and health in the elderly. Nature. 488: 178–84.

Filbey KJ, Grainger JR, Smith KA, Boon L, van Rooijen N, Harcus Y, Jenkins S, Hewitson JP, Maizels RM. 2014. Innate and adaptive type 2 immune cell responses in genetically controlled resistance to intestinal helminth infection. Immunol Cell Biol. 92: 436-448.

Finch CE, Crimmins EM. 2004. Inflammatory exposure and historical changes in human life-spans. Science. 305: 1736-1739.

Finlay CM, Walsh KP, Mills KHG. 2014. Induction of regulatory cells by helminth parasites: exploitation for the treatment of inflammatory diseases. Immunol Rev. 259: 206-230.

Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev. 128: 92–105.

Gaillard J.M., Lemaitre J.F. 2017. The William's legacy: A critical reappraisal of his nine predictions about the evolution of senescence. Evolution. 71: 2768-2785.

Goldberg EL, Dixit VD. 2015. Drivers of age-related inflammation and strategies for healthspan extension. Immunol Rev. 265: 63–74.

Grencis RK. 2015. Immunity to helminths: Resistance, regulation, and susceptibility to gastrointestinal nematodes. Ann Rev Immunol. 33: 201–25.

Grosbellet E, Zahn S, Arrivé M, Dumont S, Gourmelen S, Pévet P, Challet E, Criscuolo F. 2015. Circadian desynchronization triggers premature cellular aging in a diurnal rodent. FASEB J. 29: 4794-4803.

Guivier E, Bellenger J, Sorci G, Faivre B. 2016. Helminth interaction with the host immune system: short-term benefits and costs in relation to the infectious environment. Am Nat. 188: 253-263.

Guo W, Li M, Bhasin S. 2014. Testosterone supplementation improves anemia in aging male mice. J Gerontol A Biol Sci Med Sci. 69: 505–513.

Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. 2004. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood. 104: 2263–2268.

Hamilton WD. 1966. The moulding of senescence by natural selection. J Theor Biol. 12: 12-45.

Hang L, Blum AM, Setiawan T, Urban JP Jr, Stoyanoff KM, Weinstock JV. 2013. Heligmosomoides polygyrus bakeri infection activates colonic Foxp3(+) T cells enhancing their capacity to prevent colitis. J Immunol. 191: 1927-1934.

Helmby H. 2009. Gastrointestinal nematode infection exacerbates malaria-induced liver pathology. J Immunol. 182: 5663-5671.

Honda K, Littman DR. 2016. The microbiota in adaptive immune homeostasis and disease. Nature. 535: 75-84.

Ilmonen P, Kotrschal A, Penn DJ. 2008. Telomere attrition due to infection. PLoS ONE. 3: e2143.

Johnston MJ, MacDonald JA, McKay DM. 2009. Parasitic helminths: a pharmacopeia of anti-inflammatory molecules. Parasitology 136: 125-147.

Jones CI. 2016. Platelet function and ageing. Mamm Genome. 27: 358–366.

Khan I, Agashe D, Rolff J. 2017. Early-life inflammation, immune response and ageing. Proc R Soc B. 284: 20170125.

Kirkwood TBL, Austad SN. 2000. Why do we age? Nature. 408: 233–238.

Langille MGI, Meehan CJ, Koening JE, Dhanani AS, Rose RA, Howlett SE, Beiko RG. 2014. Microbial shifts in the aging mouse gut. Microbiome. 2: 50.

Lemaitre JF, Berger V, Bonenfant C, Douhard M, Gamelon M, Plard F, Gaillard JM. 2015. Early-late life trade-offs and the evolution of ageing in the wild. Proc R Soc B. 282: 20150209.

Lippens C, Faivre B, Sorci G. 2017. Microevolutionary response of a gut nematode to intestinal inflammation. Int J Parasitol. 47: 617-623.

Lippens C, Guivier E, Faivre B, Sorci G. 2016. Reaction norms of host immunity, host fitness and parasite performance in a mouse – intestinal nematode interaction. Int J Parasitol. 46: 133-140.

Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. 2004. Helminth parasites - masters of regulation. Immunol Rev. 201: 89–116.

Maizels RM, Hewitson JP, Murray J, Harcus YM, Dayer B, Filbey KJ, Grainger JR, McSorley HJ, Reynolds LA, Smith KA. 2012. Immune modulation and modulators in Heligmosomoides polygyrus infection. Exp Parasitol. 132: 76-89.

Maizels RM, McSorley HJ. 2016. Regulation of the host immune system by helminth parasites. J Allergy Clin Immun. 138: 666-675.

McSorley HJ, Hewitson JP, Maizels RM. 2013. Immunomodulation by helminth parasites: Defining mechanisms and mediators. Int J Parasitol. 43: 301-310.

Medawar PB. 1952. *An Unsolved Problem of Biology*. H.K. Lewis & Co., London.

Medzhitov R. 2008. Origin and physiological roles of inflammation. Nature. 454: 428-435.

Muezzinler A, Zaineddin AK, Brenner H. 2013. A systematic review of leukocyte telomere length and age in adults. Ageing Res Rev. 12: 509-519.

Okin D, Medzhitov R. 2012. Evolution of inflammatory diseases. Curr Biol. 22: R733–R740.

O'Toole PW, Jeffery IB. 2015. Gut microbiota and aging. Science. 350: 1214-1215.

Pawelec G, Goldeck D, Derhovanessian E. 2014. Inflammation, ageing and chronic disease. Curr Opin Immunol. 29: 23–28.

Penninx BW, Pahor M, Cesari M, Corsi AM, Woodman RC, Bandinelli S, Guralnik JM, Ferrucci L. 2004. Anemia is associated with disability and decreased physical performance and muscle strength in the elderly. J Am Geriatr Soc. 52: 719–724.

Pursall ER, Rolff J. 2011. Immune responses accelerate ageing: proof-of-principle in an insect model. PLoS ONE. 6: e19972.

Rook GAW. 2009. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology. 126: 3-11.

Schmid-Hempel P. 2008. Parasite immune evasion: a momentous molecular war. Trends Ecol Evol. 23: 318-326.

Schroeder B, Backhed F. 2016. Signals from the gut microbiota to distant organs in physiology and disease. Nature Med. 22: 1079-1089.

Setiawan T, Metwali A, Blum AM, Ince MN, Urban JF Jr, Elliott DE, Weinstock JV. 2007. Heligmosomoides polygyrus promotes regulatory T-Cell cytokine production in the murine normal distal intestine. Infect Immun. 75: 4655-4663.

Simons MJP. 2016. Questioning causal involvement of telomeres in aging. Ageing Res Rev. 24: 191-196.

Sorci G, Guivier E, Lippens C, Faivre B. 2016. Microbes, Parasites and Immune Diseases. *Evolutionary Thinking in Medicine. From Research to Policy and Practice*, eds Alvergne A, Jenkinson C, Faurie C (Springer, Heidelberg), pp. 211-223.

Sutton TL, Zhao A, Madden KB, Elfrey JE, Tuft BA, Sullivan CA, Urban JF Jr, Shea-Donohue T. 2008. Anti-inflammatory mechanisms of enteric Heligmosomoides polygyrus infection against trinitrobenzene sulfonic acid-induced colitis in a murine model. Infect Immun. 76: 4772-4782.

van de Berg PJ, Griffiths SJ, Yong SL, Macaulay R, Bemelman FJ, Jackson S, Henson SM, ten Berge IJ, Akbar AN, van Lier RA. 2010. Cytomegalovirus infection reduces telomere length of the circulating T cell pool. J Immunol. 184: 3417-3423.

van den Biggelaar AH, De Craen AJ, Gussekloo J, Huizinga TW, Heijmans BT, Frölich M, Kirkwood TB, Westendorp RG. 2004. Inflammation underlying cardiovascular mortality is a late consequence of evolutionary programming. FASEB J. 18:1022-1024.

Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, Listi F, Nuzzo D, Lio D, Caruso C. 2007. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev. 128: 83–91.

Williams GC. 1957. Pleiotropy, natural selection and the evolution of senescence. Evolution. 11: 398–411.

Zhang J, Rane G, Dai X, Shanmugam MK, Arfuso F, Samy RP, Lai MK, Kappei D, Kumar AP, Sethi G. 2016. Ageing and the telomere connection: An intimate relationship with inflammation. Ageing Res Rev. 25: 55-69.

Table 1. Cox regression model exploring the effect of *H. polygyrus* infection and LPS challenge on time dependent mortality in CBA mice, with early dead individuals censored. The interaction between the two treatments was not statistically significant and was removed from the model.

Sources of variation					
	Parameter ± SE	χ^2	*df*	*p*	*Hazard ratio*
H. polygyrus infection	0.53 ± 0.21	6.54	1	0.0106	1.692
LPS challenge	0.46 ± 0.20	5.11	1	0.0239	1.587

Table 2. PERMANOVA exploring the effect of *H. polygyrus* infection and LPS challenge on dissimilarity metrics of the gut microbiota. The model included *H. polygyrus* infection, LPS challenge and the interaction between the two treatments. The terms were added sequentially and the estimation of pseudo F values was based on 10,000 permutations.

		Jaccard			**Bray-Curtis**			**Unweighted Unifrac**			**Weighted Unifrac**		
Sources of variation	*df*	*Pseudo F*	R^2	*p*	*Pseudo F*	R^2	*P*	*Pseudo F*	R^2	*p*	*Pseudo F*	R^2	*p*
H. polygyrus infection	1	1.18	0.050	0.027	1.04	0.045	0.375	1.22	0.052	0.078	1.19	0.053	0.270
LPS challenge	1	1.25	0.053	0.005	0.83	0.036	0.613	1.13	0.048	0.180	0.56	0.025	0.753
H. polygyrus infection x LPS challenge	1	1.05	0.045	0.235	0.85	0.037	0.587	1.06	0.045	0.287	0.52	0.023	0.798

Table 3. Generalized linear mixed model exploring the effect of *H. polygyrus* infection and LPS challenge on changes in body mass with age. The model included squared age to model the non-linear relationship between mass and age, and age at death to model a possible effect of selective disappearance. The model also included mouse identity to take into account the repeated nature of the data. We present the minimal adequate model where non-significant interactions had been dropped (the results of the full model can be found in the supplementary material). For *H. polygyrus* infection, the negative value of the estimate indicates that non-infected mice had lower body mass compared to infected individuals. For LPS challenge, the positive value of the estimate indicates that LPS injected mice had higher body mass compared to PBS individuals, but this difference was not statistically significant.

Sources of variation				
	Estimate ± *SE*	*F*	*df*	*p*
Age	3.615 ± 0.417	75.07	1,248	<0.0001
Squared age	-0.101 ± 0.010	107.23	1,248	<0.0001
H. polygyrus infection	(non infected) -1.700 ± 0.835	4.15	1,96.5	0.0444
LPS challenge	(LPS) 0.964 ± 0.827	1.36	1,96.9	0.2465
Age at death	0.001 ± 0.004	0.09	1,105	0.7674
Random factor		*Z*		
Mouse identity	14.37 ± 2.30	6.26		<0.0001

Figure legends

Figure 1. Lifespan and age-dependent survival of CBA mice infected with *Heligmosomoides polygyrus* (or non infected) and exposed to a LPS challenge (or treated with PBS). A) Lifespan of mice in the four groups over the entire experimental period (mean ± se), lifespan was affected by the interaction between the two treatments; B) lifespan of mice in the four experimental groups once individuals that experienced early mortality had been removed (mean ± se), in this case lifespan was significantly affected by both treatments; C) age-dependent survival of mice in the four experimental groups (empty dots refer to censored individuals that died soon after the LPS challenge), survival was significantly reduced in infected compared to non infected and in LPS compared to PBS treated mice.

Figure 2. A) Red blood cell count (x $10^6/mm^3$), and B) platelet count (x $10^3/mm^3$) at the age of 17 months in CBA mice in each of the four experimental groups (mean ± se). *H. polygyrus* infected mice had significantly lower values of both red blood cell and platelet counts compared to non infected individuals.

Figure 3. Eosinophil count (% of total leukocytes) at the age of 17 months in CBA mice in each of the four experimental groups (mean ± se). *H. polygyrus* infected mice had significantly higher eosinophil counts compared to non infected individuals.

Figure 4. Relative telomere length at the age of 24 months in CBA mice in each of the four experimental groups (mean ± se). *H. polygyrus* infected mice had significantly longer relative telomere length compared to non infected individuals.

Figure 5. A) Relative abundance of bacterial families in fecal microbiota of mice in the different experimental groups averaged on 1,000 rarefied tables. B) Relative abundance of

Lachnospiraceae (mean ± se). *H. polygyrus* infected mice had a significantly lower relative abundance of *Lachnospiraceae* compared to non infected individuals.