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REVIEW

Immunity and aging: the enemy within?

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Summary

Functional analyses of changes in the immune response indicate that aging is associated with a decline of adaptive immunity whereas innate immunity is ramped up. Gene expression studies also support age-dependent changes in immunity. Studies using a large panel of methodologies and multiple species show that some of the most dramatic transcriptional changes that occur during aging are associated with immunity. This observation leads to two fundamental questions: (1) Why is the immune response altered with age? (2) Is this a consequence of aging or does it contribute to it? The origin of these changes and the mechanistic relationship among them as well as with aging must be identified. In mammals, this task is complicated by the interdependence of the innate and adaptive immune systems. The value of invertebrates as model organisms to help answer these questions is presented. This includes a description of the immune response in invertebrate models and how it compares with vertebrates, focusing on conserved pathways. Finally, these questions are explored in light of recent reports and data from our laboratory. Experimental alterations of longevity indicate that the differential expression of immunity-related genes during aging is linked to the rate of aging. Long-lived nematodes are more resistant to pathogens and blocking the expression of immune-related genes can prevent lifespan extension. These observations suggest that the immune response has a positive effect on longevity, possibly by increasing fitness. By contrast, it has been reported that activation of the immune system can reduce longevity upon starvation. We also observed that deregulation of the immune response has drastic effects on viability and longevity in *Drosophila*. These data suggest that the immune response results in a trade-off between beneficial and detrimental effects that might profoundly affect the aging process. Given this, immunity may be an ally early in life, but turns out to be an enemy as we age.

Key words aging; C. elegans; drosophila; immunity; invertebrates

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Functional changes in immunity during aging

The ever-present threat posed by infectious micro-organisms has made immune responsiveness an essential feature across diverse phyla. Not only is it predicated on the ability to distinguish self from non-self, the response must also be timely and effective. In mammals, immunity has evolved into what is commonly described as a two-component system, consisting of adaptive and innate immunity. Adaptive immunity combines highly specific antigen recognition and memory through genetic modification of lymphocytes and clonal expansion, respectively. However, this adaptive response is too slow to control an acute infection by a previously unknown antigen. By contrast, innate immunity is an immediate and fast response that can also guide aspects of the adaptive response. An overwhelming number of reports in the literature indicate that aging is correlated with a decline in immune functions. Immune deficiencies are associated with pathologies, many of which increase in frequency with age. The elderly suffer increased morbidity and mortality associated with infection, have a reduced capacity to generate high-affinity antibodies in response to vaccination and are more likely to develop select cancers and autoimmune disorders. Coincident with these functional declines are systemic changes: the adaptive system undergoes remodelling, the innate system is up-regulated and chronic inflammation develops.

Adaptive immunity

Adaptive immunity relies on three types of lymphocytes: B cells, cytotoxic T cells and helper T cells. B cells supply the body with antibodies, which, upon binding to their specific epitopes on an invader, facilitate its destruction. Cytotoxic T-cell membranes carry receptors to identify and mediate the killing of infected host cells harbouring surface antigens belonging to the infective agent. Helper T cells assist B cells and cytotoxic T cells through direct interactions, and also by releasing cytokines that attract and stimulate the proliferation of immune cells. Helper T cells rely on antigen-presenting cells (APCs) to process and display antigens in a form they can recognize. APCs trap antigens and break them down into smaller fragments that are transported to the cell surface bound to major histocompatibility complex (MHC)

molecules to allow recognition. The memory aspect of adaptive immunity is based on the maintenance of a small segment of memory cells from the large pool of effector cells initially generated from naïve cells in response to infection. Should a foreign antigen reappear, dormant memory cells proliferate and become activated into effector cells, thereby responding faster than naïve cells did during the first encounter. The decline of immune function with age could result from a decreased number of functional immune cells as well as the alteration of any step involved in the adaptive response. Analysis of the number and type of immune cells from organisms of different age or different longevity (because of genetic mutations or manipulations such as caloric restriction), combined with the characterization of immunologically relevant phenotypes of these cells, has been extensively used to address this issue. Because of the complexity of the immune system and the multitude of experimental systems, contradictory results are often reported. Nevertheless, some consensuses are apparent.

The lymphocyte populations that orchestrate adaptive immunity are altered by age. Aging is associated with a reduced proportion of naïve T cells relative to their memory counterparts (Miller, 1996; Chakravarti & Abraham, 1999). This could result from an increased number of memory cells because of lifelong exposure to foreign antigens and/or a reduced production of naïve cells. The latter is not supported by recent findings that, despite its involution during aging, the thymus is able to support adequate T lymphopoiesis late in life (Douek *et al*., 1998; Jamieson *et al*., 1999). The importance of the naïve/memory ratio is suggested by several studies. Homeostasis of the immune system requires that expanded populations of T cells undergo apoptosis after an infection has been resolved. Calorically restricted animals maintain apoptotic levels and naïve T-cell numbers while benefiting from lifespan extension, increased viral immunity and reduced tumour incidence (Fernandes *et al*., 1997; Spaulding *et al*., 1997; Chen *et al*., 1998). Furthermore, long-lived Snell dwarf mice do not show elevated levels of memory cells (Flurkey *et al*., 2001). More generally, the relative proportion of T-cell subsets, defined by the presence of specific membrane-associated proteins, is a critical parameter that can predict longevity. Mice that exhibit low levels of CD4 and CD8 memory cells, high levels of CD4 naïve cells and low levels of CD4/P-glycoprotein cells at mid-age live significantly longer (Miller, 2001).

In addition to shifts in population types, immune cells also exhibit reduced proliferative responses, altered cytokine production and responsiveness, diminished antigen recognition and signal transduction defects (Miller, 1996, 2000; Chakravarti & Abraham, 1999; Weksler, 2000; Szakal *et al*., 2002; McGlauchlen & Vogel, 2003). Lymphocytes in the elderly have a diminished proliferative response to mitogenic stimulation. Reduced proliferative capacity among the memory cell pool should manifest itself functionally in a diminished response to previously encountered immunological challenges. Consistent with this prediction, older animals are less efficient in fighting off recurrent infections despite a surplus of memory cells. However, this observation needs to be considered cautiously because a given memory cell could divide faster than the average memory cell, which can diminish immune capacity by reducing the set of antigens that can be totally eliminated. Stronger evidence is provided by the preserved proliferative response in rats under caloric restriction (Fernandes *et al*., 1997), in long-lived mice (Flurkey *et al*., 2001) and in very old humans (Lesourd & Meaume, 1994).

By influencing the set of cytokines produced and their corresponding receptors, aging can profoundly affect immune function. Most studies report an age-related decline of interleukin-2 (IL-2) signalling that stimulates T-cell division and consequently cell-based immunity (Chakravarti & Abraham, 1999). In old animals, the proportion of cells able to produce IL-2 decreases but the amount of mRNA expressed in those cells is not affected. Similarly, the proportion of cells carrying IL-2 receptors decreases but the number of receptors per cell and the affinity of the receptors for the ligand do not change. It is also generally accepted that aging is associated with a shift of cytokine profiles in helper T cells from the Th1 [interferon-γ (IFN-γ) and IL-2] to the Th2 (IL-4, IL-5, IL-6, IL-10 and IL-13) type. These observations point toward the importance of age-related changes in the proportion of immune cell subsets as mentioned previously, more so than a deterioration of the signalling mechanisms. However, conflicting results are observed for other cytokines and because the immune response requires a wide range of cytokines and a perfect balance between them to function optimally, this issue remains unresolved.

The correct recognition of an infectious antigen is the most crucial task performed by the immune system. The binding of MHC/peptide to a T-cell receptor initiates T-cell activation characterized by intracellular events, including the trigger of phosphorylation/dephosphorylation cascades, modification of calcium concentrations, reorganization of the cytoskeleton, transcription of specific genes and cell division (Alberola-Ila *et al*., 1997; Acuto & Cantrell, 2000; Lewis, 2001). Within the first few minutes of T-cell activation, there is increased activity of a variety of protein and lipid kinases. Abundant studies have described age-dependent alterations in the activity of these kinases as well as defects in both their relocalization and that of their substrates (Whisler *et al*., 1996, 1999; Gorgas *et al*., 1997; Kirk *et al*., 1999; Miller, 2000; Tamir *et al*., 2000; Chakravarti, 2001). Because all intracellular events associated with T-cell activation are linked, it is not surprising that calcium mobilization and cytoskeletal reorganization are also impaired in old cells (Chakravarti & Abraham, 1999; Garcia & Miller, 2002). The antibody-mediated response is also modified with age. The dependency of B cells on helper T cells for activation has complicated efforts to isolate the direct consequences of aging on this response. The quantity of natural serum antibodies specific for foreign antigens declines with age, as does the antigen-induced antibody production in response to most vaccines (Weksler, 2000). This phenomenon is correlated with a shift in the variety of antibodies B cells generate. Overall, the total level of immunoglobulins is unchanged because of increased production of antibodies directed against autoantigens. In addition to their smaller number in the elderly, antibodies specific for foreign antigens belong to different isotypes and/or display reduced affinity

(Weksler, 2000; McGlauchlen & Vogel, 2003). It remains unclear if these changes are a direct consequence of an age-related modification of B cells or an indirect consequence related to APCs. The latter is supported by recent studies consistent with reduced antigen trapping and presentation by old follicular dendritic cells (Aydar *et al*., 2002; Szakal *et al*., 2002).

Innate immunity

The innate immune system is a diverse collection of host defences, classified together on the basis that they lack specificity. As such, it includes physical and chemical barriers, reflexes, cellular components and local responses including inflammation and production of antibacterial peptides. Although it is convenient to address innate and adaptive immunity separately, the interplay between them is the key to the optimal defence of the organism against pathogens (Fearon & Locksley, 1996; Medzhitov & Janeway, 1997). Phagocytosis is facilitated by antibody-mediated opsonization and communication between the two is ongoing through cytokine signalling as well as cell–cell contact involved in antigen presentation. As with the adaptive system, the innate immune system is profoundly affected by age. Whereas the adaptive immune system exhibits a reduced response with age, the innate immune system ramps up, resulting in chronic inflammation. Several hypotheses can be made to account for this observation.

Old organisms are increasingly exposed to infections because of decreased efficiency of physical barriers such as the skin or the epithelium lining and the respiratory, gastrointestinal and genitourinary tracts. The importance of barriers is obvious but it still remains largely unexplored with respect to immunity. Possibly because they are not as lethal and debilitating as cardiovascular or neurological diseases, it is easy to neglect that diseases affecting the gastrointestinal tract incur the greatest increase in severity and frequency with age (Schmucker *et al*., 2001). The digestive system is the most exposed to micro-organisms (Xu & Gordon, 2003). Hundreds of different microbial species are permanently present and form a complex and dynamic ecosystem, the microbiota. Host genotype, diet and environmental factors influence the composition of this microflora. Reciprocally, this composition modifies the structure and properties of the gut (Falk *et al*., 1998). For instance, it provides enzymes not present in the host that influence food processing and consequently the molecules absorbable by the host. Important to the scope of this review, the establishment of a gut microbial ecosystem, and exposure to environmental antigens, is critical to the training of the immune system (Braun-Fahrlander *et al*., 2002; Xu & Gordon, 2003). In addition to physiological and training functions, the microbial ecosystem can act as a barrier by preventing the implantation and/or multiplication of pathogenic species (Hebuterne, 2003). The mechanisms of this barrier effect are likely to include the excretion of acids, competition for nutrients and gut niches, immunomodulation and the production of antimicrobial substances. Population shifts in the intestinal microbiota have been observed in the elderly (Hebuterne, 2003). Counting of selected cultured bacteria, 16S ribosomal abundance and cellular fatty acid profiles indicate that aging is associated with decreases in anaerobic populations as well as increases in enterobacteria (Hopkins *et al*., 2001). Protective bifidobacterial species diversity is also dramatically reduced with age (Hopkins & Macfarlane, 2002). This latter change could result in metabolic conditions favourable for the establishment of pathogenic species. The mechanisms modifying the microbiota with age remain to be identified, but anorexia, reduced pancreatic secretion and decline of anorectal functions could have significant contributions. Changes in the composition of the microbiota have been implicated in various diseases, and therefore it would not be surprising if this composition also affects aging and related pathologies. The impact of diet on microbiota predicts that caloric restriction will have significant consequences that could contribute to its anti-aging effects. Unfortunately, no studies examining this issue have been reported in the literature.

The chronic inflammation associated with aging could reflect an intrinsic innate or adaptive-related impaired ability to clear up foreign antigens totally. The interdependence of the innate and adaptive systems makes it difficult to identify the origin of this impairment. The decline in the adaptive system's ability to recognize antigens and the changes in cytokine profiles are expected to reduce the recruitment of the cellular components of the innate system in charge of killing invaders. In addition, the recognition of self-antigens will put the innate system into a vicious cycle to remove antigens that are permanently reconstituted or impossible to process. For instance, aging leads to the accumulation of oxidized, cross-linked or aggregated proteins (Grune & Davies, 2001). The proteolytic systems have limited or no ability to process these products. When a critical level is reached, such products may induce an immune response. Several reports are consistent with this hypothesis. Advanced glycation end-products (AGEs) bind to a large variety of cell-surface receptors and AGE binding proteins present on macrophages (Thornalley, 1998). The accumulation *in vivo* of Nε-(carboxymethyl)lysine (CML, one of the main advanced glycation end-product structures) serves as an immunological epitope to generate an autoantibody specific for CML (Shibayama *et al*., 1999). The response of cells to AGEs includes the increased expression of pro-inflammatory cytokines (Thornalley, 1998; Wang *et al*., 2002). After engulfment of a target by phagocytosis, the innate immune cells destroy it by several intracellular mechanisms, including release of toxic enzymes and generation of reactive oxygen species. The reduced CML accumulation observed in infection-sensitive NADPH-deficient mice unable to produce superoxide and related species (Jackson *et al*., 1995) indicates that the oxidants generated contribute to CML formation (Anderson & Heinecke, 2003). Age-dependent intrinsic innate functional decline is also possibly independent of the adaptive immune system. Despite some conflicting reports, natural killer (NK) cell activity is not greatly reduced with age (Ginaldi *et al*., 1999). By contrast, phagocytic capacity, the synthesis of reactive oxygen intermediates and the intracellular killing efficiency of neutrophils are impaired in the elderly (Ginaldi *et al*., 1999; Lord *et al*., 2001; Schroder & Rink,

2003). Similarly, aging is also associated with malfunction of macrophages (Lloberas & Celada, 2002). However, the origin of neutrophil and macrophage malfunctions remains to be elucidated.

Expression of immunity-related genes during aging

Along with the functional alteration, changes in the regulation of genes implicated in the immune response have been reported in a wide range of organisms. Using microarray technology, a series of studies have examined age-dependent changes in the skeletal muscle (Lee *et al*., 1999), brain (Lee *et al*., 2000) and heart (Lee *et al*., 2002) of mice. Unlike skeletal and cardiac muscle, in which expression of immunity-related genes was unchanged with age, several components of the immune response were altered in the brain, with a trend towards up-regulation of these genes. An additional mammalian study performed on skeletal muscle of rhesus monkeys revealed differences between mice and primates with respect to the tissue specificity of age-dependent changes in genes related to immunity (Kayo *et al*., 2001). Although many of the other transcript classes responded to aging as they did in mice muscle, components of the inflammatory/immune response were induced in aging primate muscle. Induction of immunity-related genes with age was also found in human senescent fibroblasts (Shelton *et al*., 1999). Comparable expression profiling has been performed in invertebrates. Single-gene mutations in these model systems offer a robust and reproducible intervention to extend lifespan and identify those changes in gene expression associated with altered longevity. McElwee *et al*. (2003) contrasted age-dependent changes in the gene expression profiles of long-lived *daf-2* mutants with *daf-2* mutants in which the longevity extension is prevented by a *daf-16* mutation. DAF-2 modulates longevity through DAF-16, a downstream transcription factor (Kenyon *et al*., 1993; Lin *et al*., 1997; Ogg *et al*., 1997). Clusters of genes sharing similar expression profiles were compared between *daf-2* and *daf-2/daf-16*. A cluster known to contain antibacterial proteins was generally down-regulated in the absence of the *daf-16* mutation. Because of high variability among genes within this cluster, further studies are needed to confirm this result. Murphy *et al*. (2003) used an equivalent approach in that they compared *daf-2* and *age-1/PI(3)K* long-lived mutants to wild-type and *daf2/daf-16* double mutants. This study clearly revealed that long-lived *daf-2* mutants display an induction of genes involved in infection response. These include two intestinally expressed lysosyme genes transcriptionally activated by infection as well as a saposin-like gene encoding a peptide with antibacterial properties.

A recent study using *Drosophila* has also found that aging is associated with the up-regulation of immunity-related genes (Pletcher *et al*., 2002). Consistent with this microarray study, an enhancer trap study independently revealed an age-dependent tissue-specific increase in the expression of immunity-related genes (Seroude *et al*., 2002). Two independent enhancer-trap strains displayed a dramatic tissue-specific increase in transcriptional activity. The most striking aspect of this increase is that it occurs at the middle of the adult life at the same time that the mortality rate increases exponentially (Fig. 1). Furthermore, experimental alterations of longevity show that the onset of expression always takes place at mid-life, indicating a link to the rate of aging (Seroude *et al*., 2002). Molecular analysis of these two strains demonstrates that they both report the transcriptional activity of a genomic region separating the *PGRP-LC* and *PGRP-LF* genes (Seroude *et al*., 2002). PGRP genes are evolutionarily conserved (Kang *et al*., 1998; Werner *et al*., 2000; Liu *et al*., 2001) and encode peptidoglycan recognition proteins that are necessary for the recognition of bacterial infection and the subsequent activation of the immune response (Michel *et al*., 2001; Choe *et al*., 2002; Gottar *et al*., 2002; Ramet *et al*., 2002b). RNA expression of these PGRP genes is induced selectively by bacterial infection in adult flies (De Gregorio *et al*.,

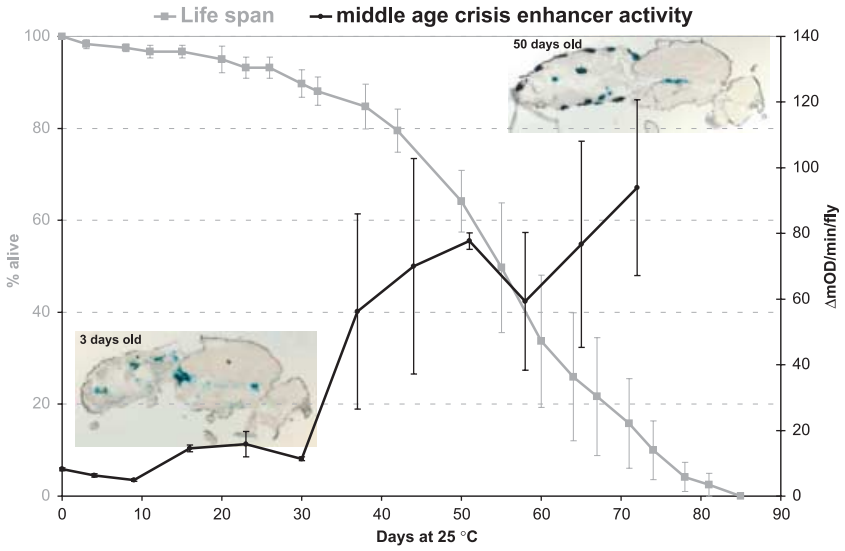

Fig. 1 The progeny from crosses between the middle-age crisis GAL4 enhancer-trap strain and a UAS-lacZ reporter strain were collected and maintained at 25 °C. The lifespan of the progeny is reported by the percentage of the population alive as a function of age (dark grey curve). Quantitative measurements of total lacZ activity (ΔmOD min^{-1} per fly) were performed using homogenized whole flies and plotted as a function of age (black curve). The spatio-temporal pattern of expression was assessed across lifespan using X-Gal staining on cryosectioned adult flies. The localization of the lacZ activity is presented in young (3 days old) and old (50 days old) animals. No expression is detected during development and an extremely low level of lacZ expression restricted to a subset of adult sensory cells until middle age (25–30 days at 25 °C). After this time the expression is induced in the abdominal oenocytes and keeps increasing until the death of the individual. The induction in the abdominal oenocytes correlates with the increase in the mortality rate. See additional data and experimental details in Seroude *et al*. (2002).

2001) and the age-dependent induction has been confirmed (Pletcher *et al*., 2002). As expected, the increased expression of the PGRP sensors is associated with the up-regulation of the downstream antimicrobial effectors (Pletcher *et al*., 2002; Seroude *et al*., 2002), indicating that aging is coupled with a global activation of the immune response.

Invertebrate immunity

At this point, it is clear that aging is associated with functional changes in the immune system correlated with changes in gene expression, cellular functions and biochemical activities. The origin of these changes and the mechanistic relationship between them, as well as with aging, will have to be identified. The experimental dissection of a network as intricate as the immune system relies on the ability to manipulate a specific component while avoiding pleiotropic effects that would complicate the interpretation of the results. In mammals, this task is complicated by the interdependence of the innate and adaptive immune systems. Invertebrates expose the seam in this graft, isolating an independent functional innate system through which immunity-related aging questions can be answered without the added layer of complexity from the adaptive system. Innate immunity is the first line of defence against infection that relies on pattern recognition receptors to recognize pathogen-derived substances. In both mammals and insects, activation of the innate immune system through these receptors results in the induction of cellular and humoral responses (Kimbrell & Beutler, 2001; Mushegian & Medzhitov, 2001; Silverman & Maniatis, 2001; Hoffmann & Reichhart, 2002; Hoffmann, 2003). The cellular response includes the stimulation of cell-based phagocytic activity, the production of reactive nitrogen and oxygen radicals and the activation of proteolytic cascades leading to blood coagulation and wound healing. The humoral response is characterized by the transcriptional activation of a panoply of genes encoding antimicrobial peptides that attack micro-organisms generally by interaction with the cellular membrane followed by displacement of lipids, alteration of membrane structure and in some cases entry of the peptide inside the target cell (Zasloff, 2002). The signalling pathways required for the activation of the innate immune responses are strikingly similar between invertebrates and humans. In both cases, infection culminates in the activation of transcription factors related to the NF-κB/Rel family.

Drosophila melanogaster

Depending on the nature of the infectious agent, *Drosophila* uses two distinct immune signalling pathways (Fig. 2). The Toll pathway is activated in response to fungal and Gram-positive intruders whereas the Imd pathway responds to Gram-negative intruders (Lemaitre *et al*., 1995, 1996, 1997). The Toll pathway is initiated by circulating recognition proteins, which include the peptidoglycan recognition protein PGRP-SA encoded by the *semmelweis* gene and the Gram-negative binding protein GNBP1 encoded by the *osiris* gene (Michel *et al*., 2001; Gobert *et al*., 2003). Loss of function mutations in either gene results in impaired survival to Gram-positive infection. Reciprocally, the concomitant overexpression of these genes results in an infection-independent activation of the Toll pathway. Upon infection, PGRP-SA and GNBP1 induce a proteolytic cascade that ultimately processes the cytokine-growth factor-like polypeptide Spaetzle into a biologically active ligand of the Toll receptor. Toll is a membrane-bound receptor with an extracellular region containing leucine-rich repeats and a cytoplasmic region similar to the corresponding region of the IL-1 receptor (IL-1R) termed the TIR domain (Hashimoto *et al*., 1988). The TIR domain interacts with at least three cytoplasmic partners: MyD88, Pelle and Tube that all contain a death domain region. Additionally, MyD88 and Pelle, respectively, contain a TIR domain and a serine–threonine kinase domain. Mutations in the Toll receptor, its ligand or any of its cytoplasmic partners impair the ability to develop an immune response (Lemaitre *et al*., 1996; Horng & Medzhitov, 2001; Tauszig-Delamasure *et al*., 2002). The activation of the Toll pathway results in the phosphorylation of the ankyrin-repeat inhibitory protein Cactus, leading to its dissociation from the dorsal-related immunity factor (DIF) transcription factor and its degradation by the proteasome (Nicolas *et al*., 1998). The factors that bridge Toll activation and the phosphorylation of Cactus remain to be identified. Although Pelle kinase activity is required for Toll signalling, it is not responsible for Cactus phosphorylation (Shelton & Wasserman, 1993). Once released from Cactus inhibition, DIF relocates from the cytoplasm to the nucleus where it activates hundreds of genes (Ip *et al*., 1993; De Gregorio *et al*., 2001, 2002; Irving *et al*., 2001). These genes are involved in the production of antimicrobial peptides as well as microbial recognition, phagocytosis, melanization, coagulation, production of reactive oxygen species and wound healing. The dissection of the Toll pathway in *Drosophila* provided critical clues for mammalian immunity. Following the discovery of the role of Toll in *Drosophila* immune response, Toll was found to be conserved across evolution to induce signals through NF-κB for activating innate and adaptative immune responses in vertebrates (Medzhitov *et al*., 1997; Poltorak *et al*., 1998). This activation involves the transcription of genes encoding pro-inflammatory cytokines and co-stimulatory molecules necessary to stimulate the adaptative response as well as genes encoding antimicrobial peptides (Akira *et al*., 2001; Janeway & Medzhitov, 2002; Tsutsumi-Ishii & Nagaoka, 2002).

The Imd pathway senses Gram-negative bacteria through the membrane-associated peptidoglycan-recognition protein PGRP-LC to initiate a specific response (Choe *et al*., 2002; Gottar *et al*., 2002; Ramet *et al*., 2002b). *PGRP-LC* mutants cannot induce the Imd pathway in response to Gram-negative infections, as indicated by impaired survival, the failure to relocate the downstream relish transcription factor into the nucleus and the inability to activate the expression of antimicrobial peptides. Like the Toll pathway, additional recognition proteins are involved in the activation of the Imd pathway. Null *PGRP-LC* mutants exhibit a milder phenotype than a null *imd* mutant

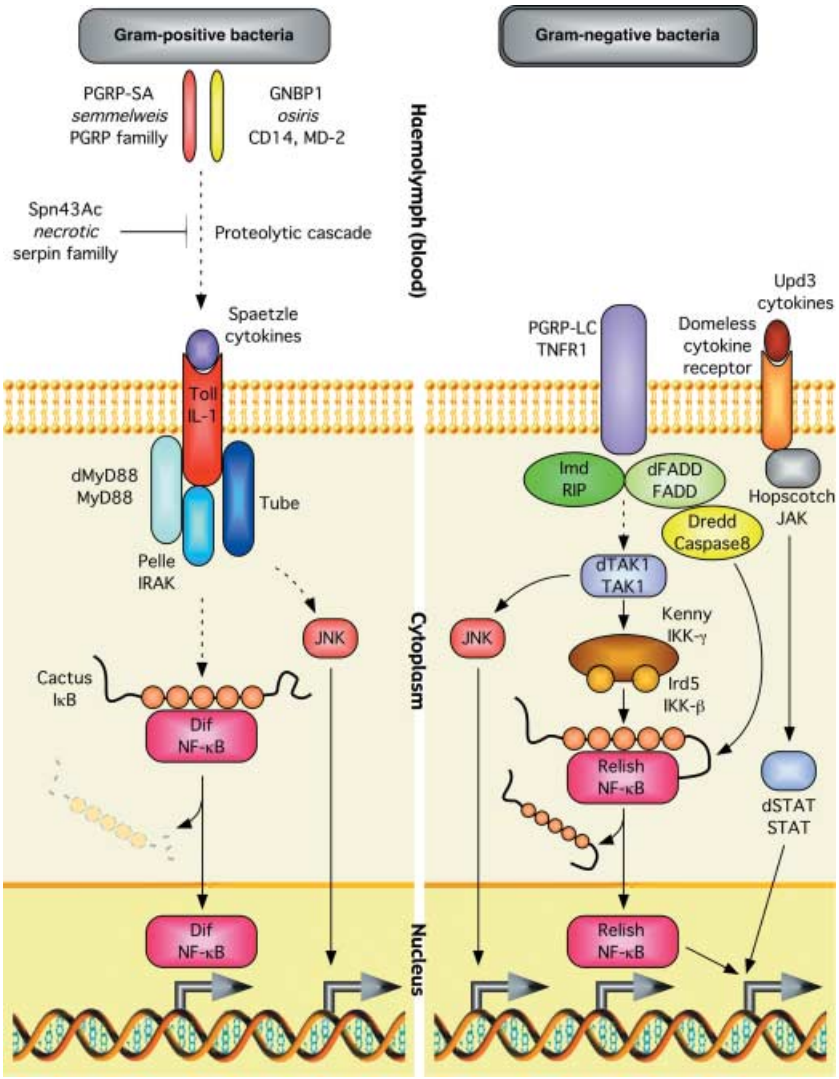

Fig. 2 *Drosophila* immune signalling pathways (see text for details).

(Hoffmann, 2003) or *dredd*, *relish* (Ramet *et al*., 2002b) and *kenny* (Gottar *et al*., 2002) mutants affecting downstream components of the pathway. These observations can be easily explained if PGRP-LC is not the sole upstream recognition factor that controls the activation of the Imd pathway. A candidate recognition protein is another peptidoglycan recognition protein, PGRP-LE (Takehana *et al*., 2002). PGRP-LE overexpression leads to a constitutive activation of the imd-mediated antibacterial response. This constitutive activation is abolished in an *imd* or *relish* mutant, indicating that PGRP-LE acts upstream of *imd* and *relish*. The molecular mechanisms between the recognition of bacteria by pattern recognition molecules and the activation of Imd remains to be elucidated. The Imd protein contains a death domain similar to that found in the mammalian tumour necrosis factor α (TNFα) receptor-interacting protein RIP but, unlike RIP, Imd does not possess a kinase domain (Georgel *et al*., 2001). Imd is necessary and sufficient to induce the transcription of antibacterial genes and, like RIP, has a dual function in controlling immune and apoptotic responses (Lemaitre *et al*., 1995; Georgel *et al*., 2001). The induction of the Imd pathway by infection leads to the phosphorylation of the NF-κB-related protein relish which, unlike Dif, is not inhibited by Cactus, and instead contains its own ankyrin-repeat inhibitory domain (Dushay *et al*., 1996). Therefore, subsequent to its phosphorylation, the nuclear translocation of relish requires endoproteolytic cleavage to release the NF-κB domain from the inhibitory domain (Stoven *et al*., 2000). The steps between Imd activation and relish translocation depend on several components genetically identified as necessary for antibacterial gene induction and resistance to Gram-negative infections. Relish phosphorylation is mediated by the IKK-β/IKK-γ kinase complex (Rutschmann *et al*., 2000; Silverman *et al*., 2000; Lu *et al*., 2001). Similar to what is observed in mammals, activation of this complex is probably achieved by the MAPKKK kinase TAK1, which is genetically acting downstream of Imd and upstream of the IKK-β/IKK-γ complex (Vidal *et al*., 2001). Relish endoproteolytic cleavage depends on the death protein dFADD, which can associate with imd through homophilic death domain interactions (Leulier

et al., 2002; Naitza *et al*., 2002) and with the caspase 8-related protein Dredd through homophilic death effector domain interactions (Hu & Yang, 2000; Leulier *et al*., 2000). Dredd interacts physically with relish and is the prime candidate for its cleavage because *Dredd* mutants cannot cleave relish and relish processing is inhibited by the caspase 8 inhibitor zVAD (Stoven *et al*., 2000, 2003). Once in the nucleus, relish regulates the expression of a wide variety of genes (De Gregorio *et al*., 2001, 2002; Irving *et al*., 2001).

Genome-wide gene expression studies suggest the existence of other signalling cascades in controlling the transcriptional response to microbial infections (De Gregorio *et al*., 2002). The JNK and JAK/STAT pathways are potential candidates (Sluss *et al*., 1996; Boutros *et al*., 2002; Agaisse *et al*., 2003). Interestingly, upon injury, the JNK pathway controls the induction of a group of genes encoding cytoskeletal proteins. This JNK-dependent response is connected to the same upstream regulatory components that control the Imd and Toll pathways. During development, the JNK pathway regulates epithelial sheet movements that are necessary for dorsal closure (Agnes & Noselli, 1999). The regulatory coupling between JNK, Imd and Toll pathways most probably coordinates antimicrobial defences and tissue repair processes. In agreement with this assumption, the JNK pathway is indeed required for efficient wound healing (Ramet *et al*., 2002a). In conjunction with the Imd pathway, the JAK/STAT pathway contributes to the regulation of the general stress factor TotA, which is probably involved in the homeostasis of damaged tissues. Activation of this pathway is dependent on the secretion of the cytokine-like upd3 by the haemocytes, indicating that the pattern recognition receptor-mediated immune response is reinforced by a general systemic immune response controlled by the release of blood cell cytokines, a process similar to the acute phase response in mammals.

Caenorhabditis elegans

At this time, *C. elegans* immunity is still an emerging field (Kurz & Ewbank, 2003). *C. elegans* is surrounded by numerous pathogens present in its soil environment, and like insects and vertebrates, upon infection, induces antibacterial defences through the transcriptional activation of many genes (Mallo *et al*., 2002). Unlike *Drosophila*, *C. elegans* does not have a cellular response. Although worms contain haemocyte-like cells, these are not actively motile and are incapable of phagocytosis (Ewbank, 2002). In addition, they are devoid of a blood-clotting ability to seal wounds. Therefore, *C. elegans* appears to rely solely on a humoral response producing antimicrobial peptides and detoxifying factors that target invaders and toxins they may release (Schulenburg *et al*., 2004). At the molecular level, analysis of its genome reveals only few components of the Toll (Toll, IRAK, Tube and IκB) and Imd (TAK1) pathways. Although *Tol1* contributes to behavioural mechanisms necessary to avoid certain pathogens, none of these components has been linked to pathogen resistance *per se* (Pujol *et al*., 2001). Moreover, the absence of pattern recognition receptors (PGRPs and GNBPs) and NF-κB-related transcription factors suggests that the Imd and Toll pathways appeared later during evolution, consistent with the more distant evolutionary relationship between nematodes and mammals than between insects and mammals (Blair *et al*., 2002). Nevertheless, *C. elegans* provides an opportunity to unveil molecular mechanisms contributing to innate immunity that could be difficult to unravel in higher organisms because of the prevalence of the Toll and Imd pathways. As in *Drosophila*, forward genetic screens for mutant worms susceptible or resistant to infections is one of the most robust methods of isolating critical components of the immune response. A screen for enhanced susceptibility to the clinical isolate of *Pseudomonas aeruginosa* strain PA14 identified the *sek-1* and *nsy-1* mutants (Kim *et al*., 2002). The *sek-1* and *nsy-1* genes are part of an evolutionary conserved p38 mitogen-activated protein kinase (MAPK) signalling pathway. In mammals, this pathway is known to mediate stress responses and the cellular immune response to bacterial lipopolysaccharide (LPS) and pro-inflammatory cytokines (Kyriakis & Avruch, 2001). The *sek-1* and *nsy-1* genes encode the upstream kinases (MAP2K and MAP3K, respectively) that lead to the activation of the p38 MAPK. Both mutants fail to phosphorylate the p38 homologue PMK-1 and as expected a *pmk-1* mutant is hypersensitive to PA14 (Kim *et al*., 2002). Importantly, the components of this pathway are also necessary for resistance to different pathogens as well as for the stress response to arsenic and acute dehydration (Kim *et al*., 2002; Aballay *et al*., 2003; Millet & Ewbank, 2004; Schulenburg *et al*., 2004). Therefore, as in mammals, the *C. elegans* p38 MAPK signalling cascade is a critical component of the immune and stress responses. The components upstream of NSY-1 that activate the cascade upon immune challenge remain to be identified. The calmodulin-dependent protein kinase II UNC-43 that activates it during neuronal development and after acute dehydration has been ruled out (Kim *et al*., 2002). Knowledge of the events downstream of PMK-1 is scarce. Following *Salmonella enterica* infection, *C. elegans* resistance requires an increased level of apoptosis in the germ cells, which is blocked by mutations in *pmk-1*, *sek-1*, *nsy-1* or genes controlling the cell death pathway (Aballay & Ausubel, 2001; Aballay *et al*., 2003). Because the increased cell death is dependent on intact *Salmonella* LPS, these data suggest that the apoptotic response lies downstream of LPS signalling and the p38 cascade. However, activation of the p38 pathway by LPS could not be demonstrated (Aballay *et al*., 2003). In addition, this response is restricted to this pathogen because it is not observed after exposure to PA14 despite being also a Gram-negative bacteria (Aballay & Ausubel, 2001). Although it is not known how the worm detects an infection, it appears that unlike *Drosophila*, the distinction between pathogens does not dictate the specificity of the immune response. In mammalian cells, LPS increases the transactivation activity of MEF2C through p38-catalysed phosphorylation (Han *et al*., 1997). *C. elegans mef-2* mutants are susceptible to infections but the connection between *pmk-1* and *mef-2* remains to be demonstrated (Alegado *et al*., 2003; Millet & Ewbank, 2004; Schulenburg

et al., 2004). Following its phosphorylation by p38, MEF2C activity depends on the interaction with Smad proteins, which are cytoplasmic signalling components of the TGF-β pathway (Quinn *et al*., 2001). Genetic analysis has shown that the *sma-2*, *sma-3* and *sma-4* genes encoding the worm Smad proteins, as well as the genes *dbl-1* and *sma-6* encoding upstream components of the TGF-β pathway (the ligand and the type I receptor, respectively), are required for infection resistance. Therefore, a molecular cross-talk is possible between the MAPK and TGF-β pathways. A recent study has also established that *C. elegans* immunity is also dependent on the *daf-2* pathway (Garsin *et al*., 2003). A molecular connection between the *daf-2* and TGF-β pathways is suggested by the identification of the *egl-4* gene encoding a homologue of the mammalian cGMP-dependent protein kinase PKG (Hirose *et al*., 2003). The *egl-4* mutants exhibit *dbl-1/sma-6*-dependent increased body size and *daf-16*-dependent lifespan extension. Unfortunately, the immune response of these mutants has not been examined. As with insects and mammals, *C. elegans* immunity relies on different signalling cascades, which have been conserved during evolution. Whereas in *Drosophila* cross-talk between pathways coordinates antibacterial defences and tissue repair, in *C. elegans* the coordination of pathways may result in an optimal response to specific pathogens. It is striking that the *daf-2* and TGF-β pathways have a dual ability to influence longevity and immunity. Molecular components capable of influencing aging and immunity could account for the relationship between aging and immunity-related changes.

Relationship between immunity and aging

The immune system is widely viewed with a narrow scope. Except with regard to combating infection, the benefits and unintended consequences of immune maintenance and activation are rarely discussed. We have documented evidence supporting age-related up-regulation of the innate system and functional declines in immunity, but such correlations only offer an entry point to establishing a relationship between immunity and aging. One possibility is that aspects of immunity that change with age are merely markers that respond to the underlying cause(s) of aging. In this scenario, age would be independent of immune status, and activation of an immune response would also be neutral in its effect. Such a relationship would make elements of the immune system valuable for tracing physiological age. To establish the direction of dependency in this relationship, systems offering a high degree of control are most valuable. To this end, aging research would be well served by simplifying its model system and avoiding unnecessary complexities. Certain changes in the adaptive system, such as increases in the frequency of autoantibodies and declines in the functionality of T cells, are of great interest therapeutically, but are unlikely to be universal causes of aging in light of how recently this system evolved. Invertebrates provide a model that is both simple and suitable. Although the question of whether invertebrate immunity has a memory-based component was raised by two recent studies (Kurtz & Franz, 2003; Little *et al*., 2003), *Drosophila* and *C. elegans* appear to rely exclusively on innate immunity. Moreover, they have: well-characterized genomes that are highly homologous to humans and are easily manipulated; potential for high-throughput applications; short generation times; biomarkers and a variety of genetic and environmental intervention strategies. Given this potential, it is tempting to perform prematurely experiments that will alter the mechanisms regulating the immune response without knowledge comparable to that for vertebrates of the progression in immune functions during aging. Vertebrate studies have raised many interesting questions. By establishing the predictive capacity that T-cell profiles have on longevity, Miller (2001) raised the questions of what influences the T-cell profile, how this effect is mediated and whether it is a marker or a determinant of aging. Manipulation of invertebrate models could answer these questions.

Like the reproductive system, the immune system could be described as a necessary evil: an acceptable trade-off in fitness made to ensure survival and propagation (Bowers *et al*., 1994; Kraaijeveld & Godfray, 1997; Lochmiller & Deerenberg, 2000). Aging and age-related diseases would be the price we pay for an immune system that defends us in youth but harms us later. It is obvious that we have to invest in an immune system that responds robustly when challenged in order to avoid the negative effects of pathogens. However, it might be possible to optimize this investment by maintaining a youthful immune system and fine-tuning it to preserve the defence properties while minimizing the detrimental consequences. Several observations support this position. Caloric restriction in mice reduces expression of immunity-related genes in both the brain and the heart (Lee *et al*., 2000, 2002). Similarly, in *Drosophila*, manipulations that extend longevity delay the up-regulation of those genes (Pletcher *et al*., 2002; Seroude *et al*., 2002). Consistent with the notion of a trade-off, immune function declines in response to increased sexual activity, and metabolism is raised in response to an immune challenge (Siva-Jothy *et al*., 1998; McKean & Nunney, 2001). The mechanism mediating this balance is unknown, but the results mentioned with regard to *C. elegans* immunity make it tempting to speculate that the insulin and TGF-β pathways, having roles in both metabolism and immunity, are involved. Whether activation of the immune system could itself be detrimental to fitness and significantly affect the aging of an organism is an additional question. Bumblebee workers under starvation exhibit a reduced lifespan when they are injected with lipopolysaccharides or microlatex beads that induce the activation of the immune system (Moret & Schmid-Hempel, 2000). In *Drosophila*, many mutants defective in immunity are available and viable, but none has been examined with respect to aging and our laboratory is currently filling this gap. However, we found that the constitutive activation of the immune response by ubiquitous over-expression of the pattern recognition protein PGRP-LC is lethal during development (our unpublished data). The same effect is mentioned in a study of PGRP-LE (Takehana *et al*., 2002). Interestingly, this effect is tissue-dependent because the targeting of over-expression to fat body or muscles is associated

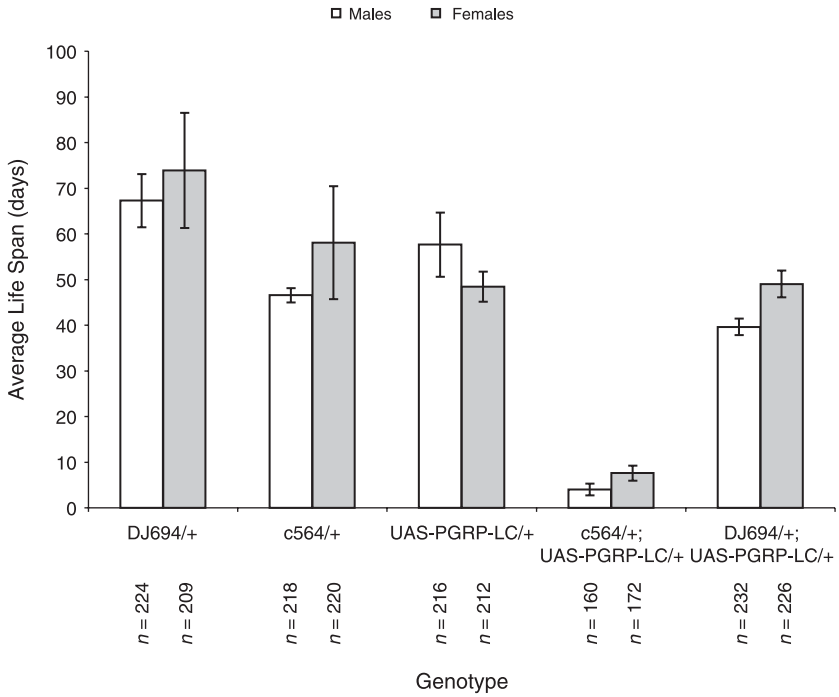

Fig. 3 Over-expression of PGRP-LC. Controls (DJ694/+; c564/+ and UAS-PGRP-LC/+) are the progeny of *Drosophila* w1118 strains carrying the DJ694 (muscle-specific GAL4 driver), c564 (fat-specific GAL4 driver) or UAS-PGRP-LC outcrossed to the w1118 parental strain. Experimental conditions are the progeny of crosses between c564 and UAS-PGRP-LC (c564/+; UAS-PGRP-LC/+) or between DJ694 and UAS-PGRP-LC (DJ694/+; UAS-PGRP-LC/+). The longevity was measured at 25 °C. Four sets of 25–30 individuals are transferred to new medium every 2 days and dead animals are scored. These data represent the average of two independent experiments. *n* is the total number of individuals tested. Error bars cover two standard deviations.

with an extreme reduction or absence of developmental lethality. This allows the dissection of the tissues in which immune function or deregulation is detrimental to longevity. Constitutive immune activation in the fat body leads to a dramatic reduction in lifespan, whereas its effect on longevity is sex dependent and less substantial when expressed in muscles (Fig. 3). Similar approaches in *Drosophila* are consistent with the concept that every single event associated with aging has different consequences as a function of the cell type. The enhancement of oxidative defences in the nervous system extends longevity but no effect is observed when this is applied in muscles (Parkes *et al.*, 1999). The reduction of programmed cell death in muscles can almost double longevity but no effect is observed when it is performed in the nervous system (our unpublished data). The molecular mechanisms underlying tissue-specific effects detrimental to the organism remain to be identified. The detrimental aspect reflects the disruption of normal cellular processes but the tissue-specificity suggests that the organism can detect localized modifications, integrate them and induce differential responses dictated by the nature of the tissue and the damage. The problem is complicated by the difficulty in pinpointing the nature and potency of damaging agents. This review has provided some clues concerning this issue. Activated blood cells produce intermediates that are known to be injurious to the host, including reactive oxygen species, glycation products and autoantibodies. The issue of glycation products is exacerbated by the body's inability to clear them. Efforts to do so compound the problem by generating autoantibodies and additional intermediates, including glycation products themselves. Overall detrimental effects do not preclude the possibility that elements of the immune response are inert or beneficial with regard to aging, but they do confirm that some aspect – whether it be fitness trade-offs or direct harm to the host – is deleterious. Detrimental effects might also explain why cohorts under caloric restriction benefit from lower production of immune-related genes, consistent with centenarian studies that associate anti-inflammatory cytokines with longevity (Bonafe *et al.*, 2001; Lio *et al.*, 2002, 2003). This area of research would benefit from studies on cohorts that are missing components of their immune system to narrow the cause of the detrimental effects. Obviously, such studies will have to be done in the absence of pathogens, a task easier to achieve in invertebrate than in vertebrate models. The developmental viability of immunity mutants and adult-specific detrimental effects of over-expression mentioned above raises another consideration. In addition to tissue-specific issues, the immune response may elicit variable results depending on the life-stage of the organism. Conditional studies, wherein select components of the immune system can be over-expressed, repressed or deleted, will be particularly informative routes to determining the biological consequences of having an immune system.

What of the more intuitive possibility that possessing or activating an immune response is beneficial in the long run? This possibility, consistent with the functional decline reviewed previously and the well-preserved immune functions observed in centenarians (Franceschi *et al.*, 1995), fuelled immunological theories of aging. Longevity is by no means the only measure of successful aging with which are associated many other aspects that would fall under the 'quality of life' umbrella. In addition, this measure is blind to the multiple events occurring during life that influence longevity. However, longevity is an important component that is often used because of the ease with which it is

recorded. At the time of writing, longevity remains the only measure used in invertebrate models because, as previously mentioned, it is far behind those available for vertebrate models in the description of the physiological, behavioural and anatomical changes taking place during aging. Fortunately, recent reports aim to fill this gap (Cook-Wiens & Grotewiel, 2002; Garigan *et al*., 2002; Herndon *et al*., 2002). In terms of longevity, the immune system could be influential in three ways. First, it might prevent death caused by infections, and in doing so increase the average longevity of a cohort. A related alternative is that the immune system might limit the accumulation of pathogen-related damage that contributes to endpoints. Finally, it may confer a benefit best summarized by the proverbial 'whatever doesn't kill you makes you stronger' and well illustrated by adaptive immunity. Any study aimed at determining the role of immunity in mediating longevity would have to associate lifespan measurement with lifelong assessment of the 'health' of various tissues. One example, which illustrates the logic, is an experiment showing that in an *age-1* background, tissue-specific expression of *age-1* in muscles restores low intestinal fat level but the animal remains long-lived (Wolkow *et al*., 2000). The implication in this case is that the defect is itself not a causative agent, and may be the by-product of the responsible process. A comparable approach following biomarkers of the immune system coincident with those related to senescent phenotypes should prove instructive in distinguishing the prospective relations between immunity and longevity. In such studies, the ability to diagnose 'cause of death' would be invaluable. Groundwork to this end is already being laid. During *C. elegans* aging, *E. coli* acquired from their diet proliferate and build up in the pharynx and intestine, leading to constipation and death (Garigan *et al*., 2002). Extended to various pathogens, it is possible to differentiate death caused by bacterial proliferation vs. toxin-mediated mechanisms (Alegado *et al*., 2003).

That said, there is already convincing evidence that activation of an immune response can be beneficial. The up-regulation of antimicrobial genes in long-lived *daf-2* worms is involved in lifespan extension given that decreasing their expression by RNAi reduces the extension (Murphy *et al*., 2003). The *daf-2* mutants combine enhanced longevity with up to a six-fold increase in their resistance to bacterial pathogens (Garsin *et al*., 2003). The manner in which the *daf-2* pathway affects survival is clearly dependent on the type of bacteria being fed to the worms. The question of whether the insulin-mediated lifespan extension was due entirely to enhanced resistance was raised by the observation that bacteria with different pathogenicities elicited a graded response. In the presence of the common soil bacteria *Bacillus subtilis* that the worm is likely to feed on in the wild, the *daf-2* mutant exhibits a 75% lifespan extension, whereas in the presence of pathogenic bacteria the extension ranges between 110% and 514% (Garsin *et al*., 2003). This result indicates that the immune response is beneficial and becomes a dominant factor when environmental pathogens are present but the lifespan extension is also mediated by factors independent of immunity. In agreement with this interpretation, resistance to pathogens in *age-1* mutants is much lower than in *daf-2* mutants (32% vs. 55%), suggesting that *daf-2* regulates longevity in an immunity-independent manner through *age-1*, whereas a large amount of its beneficial effect in a pathogen environment is conferred by *age-1*-independent components (Garsin *et al*., 2003).

It is important to bear in mind that micro-organisms themselves complicate the determination of the positive and negative impacts of immunity. As described previously, the microbial ecosystem residing in the digestive tract is an important component of the innate system as a first barrier against pathogens. In addition, exposure to foreign antigens contributes to the training of the immune system. Independently of immunity, micro-organisms can modify the structure and properties of the digestive tract as well as the nature of the nutrients available to the host. We discovered that a reduction of longevity occurs in the absence of bacteria in the gut of *Drosophila* (T. Brummel *et al.*, unpubl. data). Interestingly, this effect is age-dependent because bacteria cannot restore normal longevity after the first 4 days of adulthood. Feeding measurements ruled out the possibility that this effect was related to a change in caloric intake. Moreover, the discovery of a mutant that only exhibits lifespan extension in the presence of bacteria suggests that the flora of the gut constitutes more than an independent barrier. Such a mutant implies that pathways associated with longevity integrate feedback from the presence of bacteria. It is tempting to speculate that the insulin pathway would be involved in this signal transduction. However, the observation that diet is unchanged in bacterial-free cohorts is inconsistent with insulin-pathway mutants that are known to respond differently to diverse nutritional environments.

In conclusion, evidence collected up to this point suggests that low levels of constitutive activity and a minimal requisite induction in response to an immune challenge are ideal for longevity. This is consistent with immunity being beneficial only to the extent that it is effectively dealing with a threat more dangerous than itself. The difficulty is that such a finding is of little value: a sterile laboratory environment may favour low constitutive activity, but a natural environment rife with parasites would not. Only science fiction can propose that a pathogen-free environment could ever be realized. It follows that the fittest organism might be whichever is most flexible and effective in managing trade-offs between aspects such as reproduction, immunity and longevity. The impact of immunity on aging is complicated further by the additional wrinkles that it varies with localization and potentially with the stage of life history. For instance, immunity might be considered a high priority before reproductive maturity is reached and then decline in its importance after this stage is reached. Mediators of such trade-offs should exist to perceive, integrate and act on both environmental cues and genetic make-up. The greatest appeal of invertebrate research may be the level of dissection that can be reached to identify universal mechanisms and how they interact to affect aging. In order to determine the nature of the relationship between aging and immunity, aging research will have to keep closer to these friends and closest to these enemies.

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References

Aballay A, Ausubel FM (2001) Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. *Proc. Natl Acad. Sci. USA* **98**, 2735–2739.

Aballay A, Drenkard E, Hilbun LR, Ausubel FM (2003) *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. *Curr. Biol.* **13**, 47–52.

Acuto O, Cantrell D (2000) T-cell activation and the cytoskeleton. *Annu. Rev. Immunol.* **18**, 165–184.

Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N (2003) Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev. Cell* **5**, 441–450.

Agnes F, Noselli S (1999) Dorsal closure in *Drosophila*. A genetic model for wound healing? *CR Acad. Sci. III* **322**, 5–13.

Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* **2**, 675–680.

Alberola-Ila J, Takaki S, Kerner JD, Perlmutter RM (1997) Differential signaling by lymphocyte antigen receptors. *Annu. Rev. Immunol.* **15**, 125–154.

Alegado RA, Campbell MC, Chen WC, Slutz SS, Tan MW (2003) Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. *Cell Microbiol.* **5**, 435–444.

Anderson MM, Heinecke JW (2003) Production of N (epsilon)-(carboxymethyl) lysine is impaired in mice deficient in NADPH oxidase: a role for phagocyte-derived oxidants in the formation of advanced glycation end products during inflammation. *Diabetes* **52**, 2137–2143.

Aydar Y, Balogh P, Tew JG, Szakal AK (2002) Age-related depression of FDC accessory functions and CD21 ligand-mediated repair of costimulation. *Eur. J. Immunol.* **32**, 2817–2826.

Blair JE, Ikeo K, Gojobori T, Hedges SB (2002) The evolutionary position of nematodes. *BMC Evol. Biol.* **2**, 7.

Bonafe M, Olivieri F, Cavallone L, Giovagnetti S, Mayegiani F, Cardelli M, Pieri C, Marra M, Antonicelli R, Lisa R, Rizzo MR, Paolisso G, Monti D, Francastii C (2001) A gender – dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. *Eur. J. Immunol.* **31**, 2357–2361.

Boutros M, Agaisse H, Perrimon N (2002) Sequential activation of signaling pathways during innate immune responses in *Drosophila*. *Dev. Cell* **3**, 711–722.

Bowers RG, Boots M, Begon M (1994) Life-history trade-offs and the evolution of pathogen resistance: competition between host strains. *Proc. R Soc. Lond. B Biol. Sci.* **257**, 247–253.

Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, Lauener RP, Schierl R, Renz H, Nowak D, Von Mutius E (2002) Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* **347**, 869–877.

Chakravarti B (2001) T-cell signaling – effect of age. *Exp. Gerontol.* **37**, 33–39.

Chakravarti B, Abraham GN (1999) Aging and T-cell-mediated immunity. *Mech. Ageing Dev.* **108**, 183–206.

Chen J, Astle CM, Harrison DE (1998) Delayed immune aging in diet-restricted B6CBAT6, F1 mice is associated with preservation of naive T-cells. *J. Gerontol. A Biol. Sci. Med. Sci.* **53**, B330–B337; discussion B338–339.

Choe KM, Werner T, Stoven S, Hultmark D, Anderson KV (2002) Requirement for a peptidoglycan recognition protein (PGRP) in relish activation and antibacterial immune responses in *Drosophila*. *Science* **296**, 359–362.

Cook-Wiens E, Grotewiel MS (2002) Dissociation between functional senescence and oxidative stress resistance in *Drosophila*. *Exp. Gerontol.* **37**, 1347–1357.

De Gregorio E, Spellman PT, Rubin GM, Lemaitre B (2001) Genome-wide analysis of the *Drosophila* immune response by using oligonucleotide microarrays. *Proc. Natl Acad. Sci. USA* **98**, 12590–12595.

De Gregorio E, Spellman PT, Tzou P, Rubin GM, Lemaitre B (2002) The Toll and Imd pathways are the major regulators of the immune response in *Drosophila*. *EMBO J.* **21**, 2568–2579.

Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF, Polis MA, Haase AT, Feinberg MB, Sullivan JL, Jamieson BD, Zack JA, Picker LJ, Koup RA (1998) Changes in thymic function with age and during the treatment of HIV infection. *Nature* **396**, 690–695.

Dushay MS, Asling B, Hultmark D (1996) Origins of immunity: *Relish*, a compound Rel-like gene in the antibacterial defense of *Drosophila*. *Proc. Natl Acad. Sci. USA* **93**, 10343–10347.

Ewbank JJ (2002) Tackling both sides of the host–pathogen equation with *Caenorhabditis elegans*. *Microbes Infect.* **4**, 247–256.

Falk PG, Hooper LV, Midtvedt T, Gordon JI (1998) Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol. Mol. Biol. Rev.* **62**, 1157–1170.

Fearon DT, Locksley RM (1996) The instructive role of innate immunity in the acquired immune response. *Science* **272**, 50–53.

Fernandes G, Venkatraman JT, Turturro A, Attwood VG, Hart RW (1997) Effect of food restriction on lifespan and immune functions in long-lived Fischer-344, x Brown Norway F1 rats. *J. Clin. Immunol.* **17**, 85–95.

Flurkey K, Papaconstantinou J, Miller RA, Harrison DE (2001) Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc. Natl Acad. Sci. USA* **98**, 6736–6741.

Franceschi C, Monti D, Sansoni P, Cossarizza A (1995) The immunology of exceptional individuals: the lesson of centenarians. *Immunol. Today* **16**, 12–16.

Garcia GG, Miller RA (2002) Age-dependent defects in TCR-triggered cytoskeletal rearrangement in CD4+ T-cells. *J. Immunol.* **169**, 5021–5027.

Garigan D, Hsu AL, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* **161**, 1101–1112.

Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, Calderwood SB, Ruvkun G, Ausubel FM (2003) Long-lived *C. elegans daf-2* mutants are resistant to bacterial pathogens. *Science* **300**, 1921.

Georgel P, Naitza S, Kappler C, Ferrandon D, Zachary D, Swimmer C, Kopczynski C, Duyk G, Reichhart JM, Hoffmann JA (2001) *Drosophila* immune deficiency (IMD) is a death domain protein that activates antibacterial defense and can promote apoptosis. *Dev. Cell* **1**, 503–514.

Ginaldi L, De Martinis M, D'Ostilio A, Marini L, Loreto MF, Quaglino D (1999) The immune system in the elderly. III. Innate immunity. *Immunol. Res.* **20**, 117–126.

Gobert V, Gottar M, Matskevich AA, Rutschmann S, Royet J, Belvin M, Hoffmann JA, Ferrandon D (2003) Dual activation of the *Drosophila* toll pathway by two pattern recognition receptors. *Science* **302**, 2126–2130.

Gorgas G, Butch ER, Guan KL, Miller RA (1997) Diminished activation of the MAP kinase pathway in CD3-stimulated T lymphocytes from old mice. *Mech. Ageing Dev.* **94**, 71–83.

Gottar M, Gobert V, Michel T, Belvin M, Duyk G, Hoffmann JA, Ferrandon D, Royet J (2002) The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. *Nature* **416**, 640–644.

Grune T, Davies KJ (2001) Oxidative processes in aging. In *Handbook of the Biology of Aging* (Masoro EJ, Austad SN, eds). San Diego: Academic Press, pp. 25–58.

Han J, Jiang Y, Li Z, Kravchenko VV, Ulevitch RJ (1997) Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. *Nature* **386**, 296–299.

Hashimoto C, Hudson KL, Anderson KV (1988) The *Toll* gene of *Drosophila*, required for dorsal–ventral embryonic polarity, appears to encode a transmembrane protein. *Cell* **52**, 269–279.

Hebuterne X (2003) Gut changes attributed to ageing: effects on intestinal microflora. *Curr. Opin. Clin. Nutr. Metab. Care* **6**, 49–54.

Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M (2002) Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* **419**, 808–814.

Hirose T, Nakano Y, Nagamatsu Y, Misumi T, Ohta H, Ohshima Y (2003) Cyclic GMP-dependent protein kinase EGL-4 controls body size and lifespan in *C. elegans*. *Development* **130**, 1089–1099.

Hoffmann JA (2003) The immune response of *Drosophila*. *Nature* **426**, 33–38.

Hoffmann JA, Reichhart JM (2002) *Drosophila* innate immunity: an evolutionary perspective. *Nat. Immunol.* **3**, 121–126.

Hopkins MJ, Macfarlane GT (2002) Changes in predominant bacterial populations in human faeces with age and with *Clostridium* difficile infection. *J. Med. Microbiol.* **51**, 448–454.

Hopkins MJ, Sharp R, Macfarlane GT (2001) Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* **48**, 198–205.

Horng T, Medzhitov R (2001) *Drosophila* MyD88 is an adapter in the Toll signaling pathway. *Proc. Natl Acad. Sci. USA* **98**, 12654–12658.

Hu S, Yang X (2000) dFADD, a novel death domain-containing adapter protein for the *Drosophila* caspase DREDD. *J. Biol. Chem.* **275**, 30761–30764.

Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, Gonzalezcrespo S, Tatei K, Levine M (1993) *Dif*, a dorsal-related gene that mediates an immune-response in *Drosophila*. *Cell* **75**, 753–763.

Irving P, Troxler L, Heuer TS, Belvin M, Kopczynski C, Reichhart JM, Hoffmann JA, Hetru C (2001) A genome-wide analysis of immune responses in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 15119–15124.

Jackson SH, Gallin JI, Holland SM (1995) The *p47phox* mouse knockout model of chronic granulomatous disease. *J. Exp. Med.* **182**, 751–758.

Jamieson BD, Douek DC, Killian S, Hultin LE, Scripture-Adams DD, Giorgi JV, Marelli D, Koup RA, Zack JA (1999) Generation of functional thymocytes in the human adult. *Immunity* **10**, 569–575.

Janeway CA Jr, Medzhitov R (2002) Innate immune recognition. *Annu. Rev. Immunol.* **20**, 197–216.

Kang D, Liu G, Lundstrom A, Gelius E, Steiner H (1998) A peptidoglycan recognition protein in innate immunity conserved from insects to humans. *Proc. Natl Acad. Sci. USA* **95**, 10078–10082.

Kayo T, Allison DB, Weindruch R, Prolla TA (2001) Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. *Proc. Natl Acad. Sci. USA* **98**, 5093–5098.

Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464.

Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, Inoue H, Tanaka-Hino M, Hisamoto N, Matsumoto K, Tan MW, Ausubel FM (2002) A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623–626.

Kimbrell DA, Beutler B (2001) The evolution and genetics of innate immunity. *Nature Rev. Genet.* **2**, 256–267.

Kirk CJ, Freilich AM, Miller RA (1999) Age-related decline in activation of JNK by TCR- and CD28-mediated signals in murine T-lymphocytes. *Cell Immunol.* **197**, 75–82.

Kraaijeveld AR, Godfray HC (1997) Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature* **389**, 278–280.

Kurtz J, Franz K (2003) Innate defence: evidence for memory in invertebrate immunity. *Nature* **425**, 37–38.

Kurz CL, Ewbank JJ (2003) *Caenorhabditis elegans*: an emerging genetic model for the study of innate immunity. *Nat. Rev. Genet* **4**, 380–390.

Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* **81**, 807–869.

Lee CK, Allison DB, Brand J, Weindruch R, Prolla TA (2002) Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc. Natl Acad. Sci. USA* **99**, 14988–14993.

Lee CK, Klopp RG, Weindruch R, Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* **285**, 1390–1393.

Lee CK, Weindruch R, Prolla TA (2000) Gene-expression profile of the ageing brain in mice. *Nat. Genet.* **25**, 294–297.

Lemaitre B, Kromermetzger E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann JA (1995) A recessive mutation, immunedeficiency (*Imd*), defines 2 distinct control pathways in the *Drosophila* host-defense. *Proc. Natl Acad. Sci. USA* **92**, 9465–9469.

Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983.

Lemaitre B, Reichhart JM, Hoffmann JA (1997) *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl Acad. Sci. USA* **94**, 14614–14619.

Lesourd BM, Meaume S (1994) Cell mediated immunity changes in ageing, relative importance of cell subpopulation switches and of nutritional factors. *Immunol. Lett.* **40**, 235–242.

Leulier F, Rodriguez A, Khush RS, Abrams JM, Lemaitre B (2000) The *Drosophila* caspase Dredd is required to resist gram-negative bacterial infection. *EMBO Rep.* **1**, 353–358.

Leulier F, Vidal S, Saigo K, Ueda R, Lemaitre B (2002) Inducible expression of double-stranded RNA reveals a role for dFADD in the regulation of the antibacterial response in *Drosophila* adults. *Curr. Biol.* **12**, 996–1000.

Lewis RS (2001) Calcium signaling mechanisms in T lymphocytes. *Annu. Rev. Immunol.* **19**, 497–521.

Lin K, Dorman JB, Rodan A, Kenyon C (1997) daf-16: An HNF-3/forkhead family member that can function to double the lifespan of *Caenorhabditis elegans*. *Science* **278**, 1319–1322.

Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafe M, Cavallone L, Franceschi C, Caruso C (2002) Gender–specific association between -1082 IL-10 promoter polymorphism and longevity. *Genes Immunol.* **3**, 30–33.

Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafe M, Cavallone L, Marchegiani F, Olivieri F, Franceschi C, Caruso C (2003) Inflammation, genetics, and longevity: further studies on the protective effects in men of IL-10–1082 promoter SNP and its interaction with TNF-alpha-308 promoter SNP. *J. Med. Genet.* **40**, 296–299.

Little TJ, O'Connor B, Colegrave N, Watt K, Read AF (2003) Maternal transfer of strain-specific immunity in an invertebrate. *Curr. Biol.* **13**, 489–492.

Liu C, Xu Z, Gupta D, Dziarski R (2001) Peptidoglycan recognition proteins: a novel family of four human innate immunity pattern recognition molecules. *J. Biol. Chem.* **276**, 34686–34694.

Lloberas J, Celada A (2002) Effect of aging on macrophage function. *Exp. Gerontol.* **37**, 1325–1331.

Lochmiller RL, Deerenberg C (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *OIKOS* **88**, 87–98.

Lord JM, Butcher S, Killampali V, Lascelles D, Salmon M (2001) Neutrophil ageing and immunesenescence. *Mech. Ageing Dev.* **122**, 1521–1535.

Lu Y, Wu LP, Anderson KV (2001) The antibacterial arm of the *Drosophila* innate immune response requires an IkappaB kinase. *Genes Dev.* **15**, 104–110.

Mallo GV, Kurz CL, Couillault C, Pujol N, Granjeaud S, Kohara Y, Ewbank JJ (2002) Inducible antibacterial defense system in *C. elegans*. *Curr. Biol.* **12**, 1209–1214.

McElwee J, Bubb K, Thomas JH (2003) Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. *Aging Cell* **2**, 111–121.

McGlauchlen KS, Vogel LA (2003) Ineffective humoral immunity in the elderly. *Microbes Infect.* **5**, 1279–1284.

McKean KA, Nunney L (2001) Increased sexual activity reduces male immune function in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **98**, 7904–7909.

Medzhitov R, Janeway CA Jr (1997) Innate immunity: impact on the adaptive immune response. *Curr. Opin. Immunol.* **9**, 4–9.

Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397.

Michel T, Reichhart JM, Hoffmann JA, Royet J (2001) *Drosophila* Toll is activated by Gram-positive bacteria through a circulating peptidoglycan recognition protein. *Nature* **414**, 756–759.

Miller RA (1996) Aging and the immune response. In *Handbook of the Biology of Aging* (Schneider EL, Rowe JW, eds). San Diego: Academic Press, pp. 355–392.

Miller RA (2000) Effect of aging on T lymphocyte activation. *Vaccine* **18**, 1654–1660.

Miller RA (2001) Biomarkers of aging: prediction of longevity by using age-sensitive T-cell subset determinations in a middle-aged, genetically heterogeneous mouse population. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**, B180–B186.

Millet AC, Ewbank JJ (2004) Immunity in *Caenorhabditis elegans*. *Curr. Opin. Immunol.* **16**, 4–9.

Moret Y, Schmid-Hempel P (2000) Survival for immunity: the price of immune system activation for bumblebee workers. *Science* **290**, 1166–1168.

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**, 277–283.

Mushegian A, Medzhitov R (2001) Evolutionary perspective on innate immune recognition. *J. Cell Biol.* **155**, 705–710.

Naitza S, Rosse C, Kappler C, Georgel P, Belvin M, Gubb D, Camonis J, Hoffmann JA, Reichhart JM (2002) The *Drosophila* immune defense against gram-negative infection requires the death protein dFADD. *Immunity* **17**, 575–581.

Nicolas E, Reichhart JM, Hoffmann JA, Lemaitre B (1998) In vivo regulation of the I kappa B homologue cactus during the immune response of *Drosophila*. *J. Biol. Chem.* **273**, 10463–10469.

Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**, 994–999.

Parkes TL, Hilliker AJ, Phillips JP (1999) Motorneurons, reactive oxygen, and lifespan in *Drosophila*. *Neurobiol. Aging* **20**, 531–535.

Pletcher SD, Macdonald SJ, Marguerie R, Certa U, Stearns SC, Goldstein DB, Partridge L (2002) Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. *Curr. Biol.* **12**, 712–723.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel CX, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi Castagnoli P, Layton B, Beutler B (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088.

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan MW, Ray KP, Solari R, Johnson CD, Ewbank JJ (2001) A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr. Biol.* **11**, 809–821.

Quinn ZA, Yang CC, Wrana JL, McDermott JC (2001) Smad proteins function as co-modulators for MEF2 transcriptional regulatory proteins. *Nucleic Acids Res.* **29**, 732–742.

Ramet M, Lanot R, Zachary D, Manfruelli P (2002a) JNK signaling pathway is required for efficient wound healing in *Drosophila*. *Dev. Biol.* **241**, 145–156.

Ramet M, Manfruelli P, Pearson A, Mathey-Prevot B, Ezekowitz RA (2002b) Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. *Nature* **416**, 644–648.

Rutschmann S, Jung AC, Zhou R, Silverman N, Hoffmann JA, Ferrandon D (2000) Role of *Drosophila* IKK gamma in a toll-independent antibacterial immune response. *Nat. Immunol.* **1**, 342–347.

Schmucker DL, Thoreux K, Owen RL (2001) Aging impairs intestinal immunity. *Mech. Ageing Dev.* **122**, 1397–1411.

Schroder AK, Rink L (2003) Neutrophil immunity of the elderly. *Mech. Ageing Dev.* **124**, 419–425.

Schulenburg H, Kurz CL, Ewbank JJ (2004) Evolution of the innate immune response: the worm perspective. *Immunol. Rev.* **198**, 36–58.

Seroude L, Brummel T, Kapahi P, Benzer S (2002) Spatio-temporal analysis of gene expression during aging in *Drosophila melanogaster*. *Aging Cell* **1**, 47–56.

Shelton DN, Chang E, Whittier PS, Choi D, Funk WD (1999) Microarray analysis of replicative senescence. *Curr. Biol.* **9**, 939–945.

Shelton CA, Wasserman SA (1993) *Pelle* encodes a protein-kinase required to establish dorsoventral polarity in the *Drosophila* embryo. *Cell* **72**, 515–525.

Shibayama R, Araki N, Nagai R, Horiuchi S (1999) Autoantibody against N (epsilon)-(carboxymethyl) lysine: an advanced glycation end product of the Maillard reaction. *Diabetes* **48**, 1842–1849.

Silverman N, Maniatis T (2001) NF-kappaB signaling pathways in mammalian and insect innate immunity. *Genes Dev.* **15**, 2321–2342.

Silverman N, Zhou R, Stoven S, Pandey N, Hultmark D, Maniatis T (2000) A *Drosophila* IkappaB kinase complex required for Relish cleavage and antibacterial immunity. *Genes Dev.* **14**, 2461–2471.

Siva-Jothy MT, Tsubaki Y, Hooper R (1998) Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. *Physiol. Entomol.* **23**, 274–277.

Sluss HK, Han ZQ, Barrett T, Davis RJ, Ip YT (1996) A JNK signal transduction pathway that mediates morphogenesis and an immune response in *Drosophila*. *Genes Dev.* **10**, 2745–2758.

Spaulding CC, Walford RL, Effros RB (1997) The accumulation of non-replicative, non-functional, senescent T-cells with age is avoided in calorically restricted mice by an enhancement of T-cell apoptosis. *Mech. Ageing Dev.* **93**, 25–33.

Stoven S, Ando I, Kadalayil L, Engstrom Y, Hultmark D (2000) Activation of the *Drosophila* NF-kappaB factor Relish by rapid endoproteolytic cleavage. *EMBO Rep.* **1**, 347–352.

Stoven S, Silverman N, Junell A, Hedengren-Olcott M, Erturk D, Engstrom Y, Maniatis T, Hultmark D (2003) Caspase-mediated processing of the *Drosophila* NF-kappaB factor Relish. *Proc. Natl Acad. Sci. USA* **100**, 5991–5996.

Szakal AK, Aydar Y, Balogh P, Tew JG (2002) Molecular interactions of FDCs with B-cells in aging. *Semin. Immunol.* **14**, 267–274.

Takehana A, Katsuyama T, Yano T, Oshima Y, Takada H, Aigaki T, Kurata S (2002) Overexpression of a pattern-recognition receptor, peptidoglycan-recognition protein-LE, activates imd/relish-mediated antibacterial defense and the prophenoloxidase cascade in *Drosophila* larvae. *Proc. Natl Acad. Sci. USA* **99**, 13705–13710.

Tamir A, Eisenbraun MD, Garcia GG, Miller RA (2000) Age-dependent alterations in the assembly of signal transduction complexes at the site of T–cell/APC interaction. *J. Immunol.* **165**, 1243–1251.

Tauszig-Delamasure S, Bilak H, Capovilla M, Hoffmann JA, Imler JL (2002) *Drosophila* MyD88 is required for the response to fungal and Gram-positive bacterial infections. *Nature Immunol.* **3**, 91–97.

Thornalley PJ (1998) Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cell Mol. Biol.* **44**, 1013–1023.

Tsutsumi-Ishii Y, Nagaoka I (2002) NF-kappa B-mediated transcriptional regulation of human *beta-defensin-2* gene following lipopolysaccharide stimulation. *J. Leukocyte Biol.* **71**, 154–162.

Vidal S, Khush RS, Leulier F, Tzou P, Nakamura M, Lemaitre B (2001) Mutations in the *Drosophila dTAK1* gene reveal a conserved function for MAPKKKs in the control of rel/NF-kappaB-dependent innate immune responses. *Genes Dev.* **15**, 1900–1912.

Wang Z, Li DD, Liang YY, Wang DS, Cai NS (2002) Activation of astrocytes by advanced glycation end products: cytokines induction and nitric oxide release. *Acta Pharmacol. Sin.* **23**, 974–980.

Weksler ME (2000) Changes in the B-cell repertoire with age. *Vaccine* **18**, 1624–1628.

Werner T, Liu G, Kang D, Ekengren S, Steiner H, Hultmark D (2000) A family of peptidoglycan recognition proteins in the fruit fly *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **97**, 13772–13777.

Whisler RL, Chen M, Liu B, Newhouse YG (1999) Age-related impairments in TCR/CD3 activation of ZAP-70 are associated with reduced tyrosine phosphorylations of zeta-chains and p59fyn/p56lck in human T-cells. *Mech. Ageing Dev.* **111**, 49–66.

Whisler RL, Newhouse YG, Bagenstose SE (1996) Age-related reductions in the activation of mitogen-activated protein kinases p44mapk/ERK1 and p42mapk/ERK2 in human T-cells stimulated via ligation of the T-cell receptor complex. *Cell Immunol.* **168**, 201–210.

Wolkow CA, Kimura KD, Lee MS, Ruvkun G (2000) Regulation of *C-elegans* lifespan by insulinlike signaling in the nervous system. *Science* **290**, 147–150.

Xu J, Gordon JI (2003) Inaugural article: honor thy symbionts. *Proc. Natl Acad. Sci. USA* **100**, 10452–10459.

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395.