AIBT

# Summer School 2015

**Alloreattività e trapianti nell'uomo:**
**le nuove metodiche di studio**
**e i trapianti alternativi**

# Infezioni da CMV, invecchiamento e longevità: ruolo dei KIR ed HLA

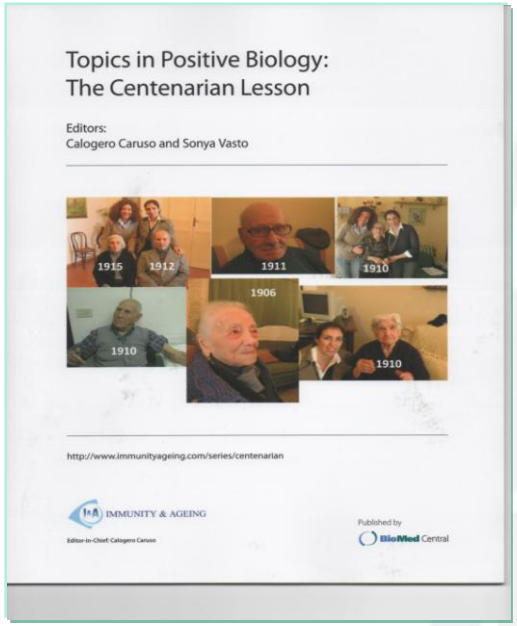
Calogero Caruso

**04 - 06 giugno 2015 Villaggio Cala la Luna Favignana (TP)**

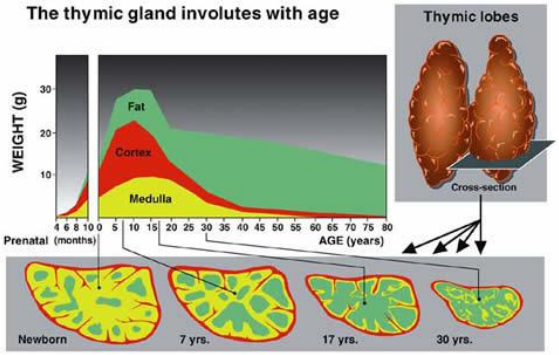
# Outline of the presentation

Ageing

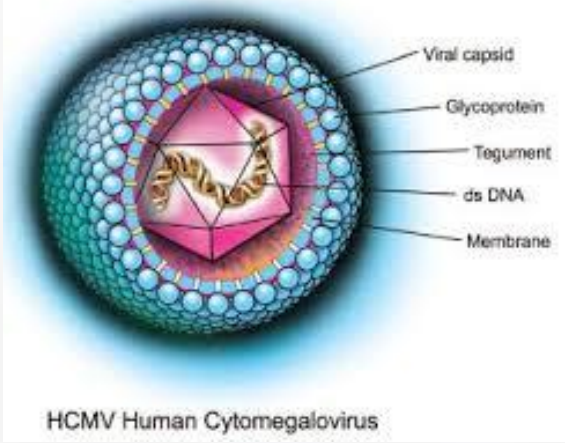

Longevity



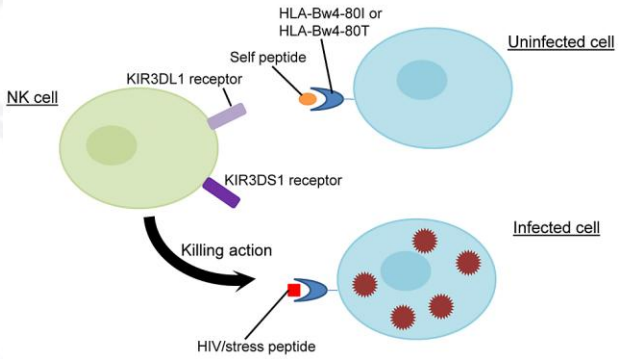

Immunosenescence




HCMV




Genetic Control




Slowing ageing

# What is ageing?

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# Ageing

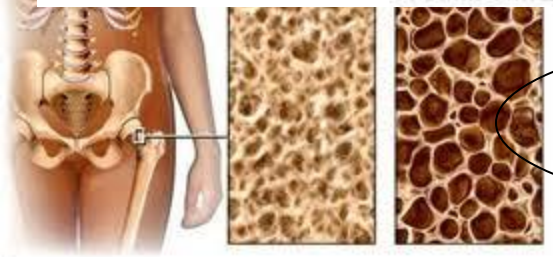
Ageing is a complex process, results from a breakdown of the system of organization of self and a reduced ability to adapt to the environment.

# Senescence

The process by which a cell loses its function and its ability to divide and grow.

## *Aging:*

***Changes in the cells (cellular senescence), tissues and organs after maturation***



***Progressive loss of physiological functions of tissues and organs***

***Reduced ability to respond to environmental stimuli, due to an ineffective homeostatic response***

- *Immune system*
- *Endocrine system*
- *Nervous system*

→ *Homeostasis* → *Health*

***Increased susceptibility to disease***

***Increased death risk***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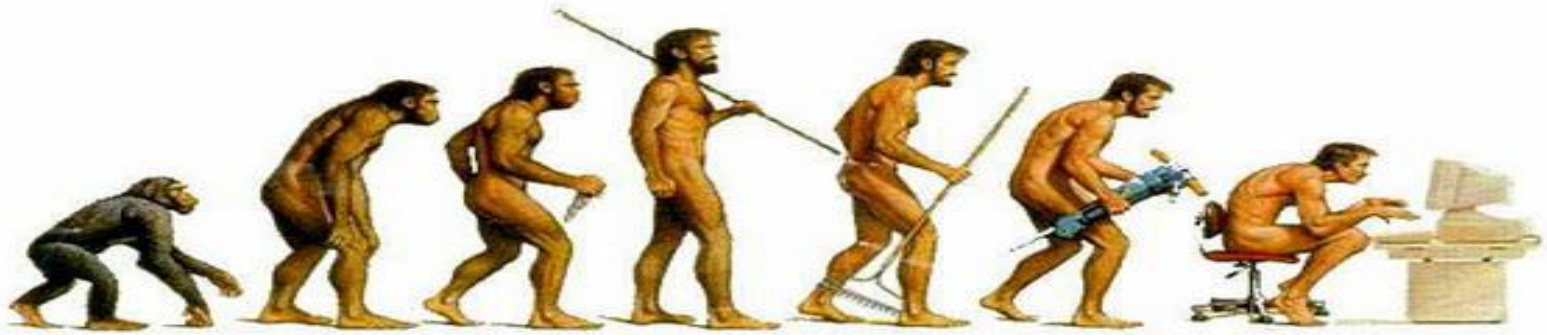
# EVOLUTION, AGEING AND LONGEVITY



**During the evolution biological molecules are developed on energy plans that allowed them to maintain the molecular fidelity until the time of reproduction. In different animal species the amount of energy required from birth to the reproduction should be kept constant. What determines longevity is the residual capacity after the reproductive period. Organisms use the resources to metabolic priorities that compete with each other: the growth, reproduction, maintenance and repair and storage.**

# What is longevity?

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# Editorial

## Ageing, Longevity, Exceptional Longevity and Related Genetic and Non Genetics Markers: Panel Statement

Peter Avery[1], Nir Barzilai[2], Athanase Benetos[3], Helen Bilianou[4], Miriam Capri[5,6], Calogero Caruso[7], Claudio Franceschi[5,6], Niki Katsiki[8], Dimitri P. Mikhailidis[9], George Panotopoulos[10], Ewa Sikora[11], Irene P. Tzanetakou[12] and Genovefa Kolovou[13,*]

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In May 2012, a group of scientists and clinicians met in Athens (Greece) to consider the relevance of ageing, longevity, exceptional longevity and related genetic and non genetic markers. During this meeting, we firstly reviewed recent epidemiological and clinical studies on ageing, longevity and exceptional longevity, briefly analyzed the ageing theories and discussed successful and unsuccessful ageing also taking into account the evolutionary perspective. Secondly, we considered the three phenotypes based on the definition of ageing, longevity and exceptional longevity and the associated biomarkers. Third, we discussed proposed treatments suitable to counteract or slow down ageing. Finally, this panel produced a consensus statement to highlight the importance of ageing, longevity and exceptional longevity, since this is a rapidly increasing phenotype worldwide. We acknowledge that not all experts in this field may completely agree with this statement.

## SUMMARY OF PANEL POSITION

1. Ageing is most likely one component of life, which first emerged in economically developed countries and results from a breakdown of self organizing system and reduced ability to adapt to the environment. Ageing processes are defined as those that amplify the vulnerability of subjects, as they become older, to the factors that finally lead to death. An emerging concept is the difference between chronological and biological ageing: tissues and organs of the same body may have a diverse rate of ageing in contrast with the chronological age of the individual, and conversely individuals with the same chronological age may have different rate of ageing and a different biological age.
2. Many variables contribute to ageing/longevity such as cultural, anthropological and socio-economic status as well sex and gender (women live longer than men) and ethnic differences (explained by discrepancies in healthcare, environmental and economic status, genetics as well as life occupation) also exist in relation to ageing/longevity, as well as stochastic events.
3. Successful ageing involves avoidance (or late onset) of age-related disease including cardiovascular disease which is the main cause of death, and other organ specific diseases, disability, preservation of desirable cognitive and physical function and social activities throughout the life span.
4. Many definitions of longevity are proposed, but at present no consensus definition has been established. On the basis of demographic data, we propose that exceptional longevity may be defined in relative and absolute terms. "Relative" suggests that longevity is concept country/population specific and must take into consideration the life expectancy of the different populations/countries, which show great variability owing to historical, anthropological and socio-economic differences. In "absolute" terms longevity could be defined according to the maximum lifespan attained and scientifically validated by human beings in the planet.
5. Familial longevity refers to families enriched by long living members. On the basis of stringent criteria and accurate analysis of the demographic data in Europe, familial longevity can be identified as that of families where at least two living members aged $\geq$ 90 years were present.
6. Most genetic studies on human longevity suffer from a variety of limitations due to the difficulty in recruiting large number of phenotypically well characterized long living people (centenarians), small cohort groups, difficulties in validation of the findings in different cohorts in order to test the general meaning of the findings, lack of controls born at the same time as centenarians but with different life span duration and lack of important information such as environmental factors, lifestyle and quality of life, presence and duration of disabilities and diseases.
7. There is evidence identifying some genes related to longevity and ageing. Such genes are included in a variety of signaling pathways, i.e. insulin/insulin-like growth factor (IGF-1), nutrient-sensing (mTOR), oxidative stress and anti-oxidants, control of immune-inflammatory responses and lipid metabolism as well as in mitochondrial DNA (mtDNA). However, more evidence is needed. In addition, it is becoming clear that epigenetic changes linked to diet or to other environmental/life style factors (physical activity, emotional stress) play a role in longevity attainment.
8. Most life-extension effects in animal models have been found to result from knocking down a relatively large number of different genes. This unexpected finding would suggest that the wild-type gene shortens lifespan. It is important to note that the animals with an extended lifespan as a consequence of genetic/environmental manipulations in laboratory conditions show a shorten lifespan when they live in environmental conditions more similar to those of real life. This can be considered "a laboratory trait" in comparison with the centenarians analyzed in studies on human longevity who spent their life in a real and often harsh environment.
9. Combination of animal genetic studies, human genetic population-based and family-based studies, as well as, "omics" studies, are approaches that may help identify genes/pathways (and also biomarkers) involved in ageing/longevity.
10. Caloric restriction, hormonal replacement and antioxidant treatments were reported to promote healthy longevity in some animal models. Also, some strategies for enhancing longevity were introduced e.g. engineered negligible senescence, nucleic acid therapy and cloning of genes related to ageing or genes which could promote longevity. Moreover, mechanisms that affect cell senescence *in vitro* and/or animal ageing may not be fully relevant to humans. Therefore, scientists would always seek definitive clinical evidence and validation of such data in humans.

This is the best way to respond to "commercial" anti-ageing medicine. Other strategies are worthwhile to pursue in humans, such as prevention of vascular events, cancer screening and healthy life style which together have the potential to decrease morbidity and mortality associated with ageing.

These statements are based on the longevity consensus documents [1-9].

# Genes and alleles associated to longevity

| Gene | Allele | Frequency in centenarians | Association to diseases | Possible role in longevity |
|---|---|---|---|---|
| APOE | E4* | Reduced | CVD<br>AD | Vascular integrity<br>Control of inflammation |
| FOXO3A | rs2802292* | Increased | - | Cellular homeostasis control |

*, Results obtained with candidate gene approach and repeated in different studies.

A genetic example of demographic selection is the reduced frequency in ε4 allele of apolipoprotein E (ApoE) in aged people. Allele frequency of APOE ε4, associated to CVD and AD, is reduced in centenarians, while prevalence of ε2 allele, is relatively high. 

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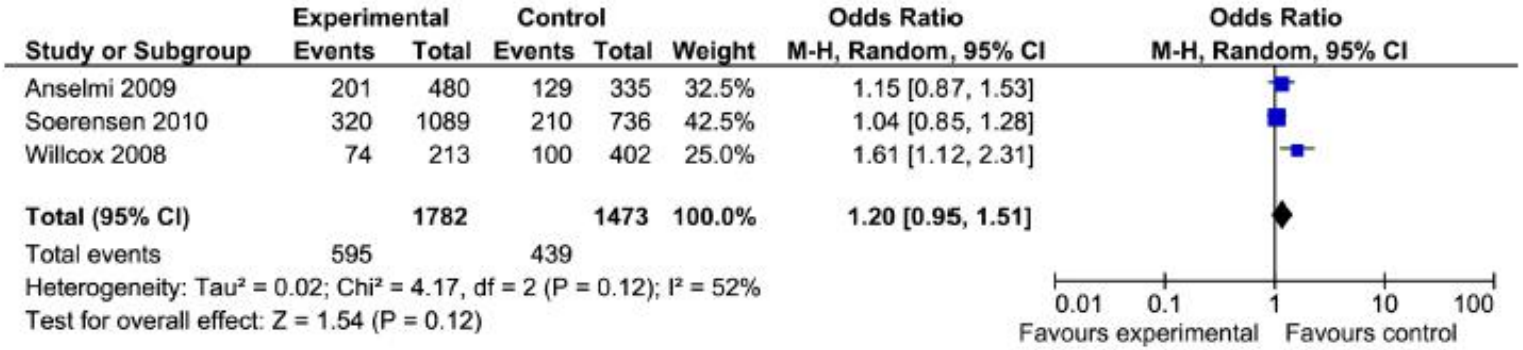
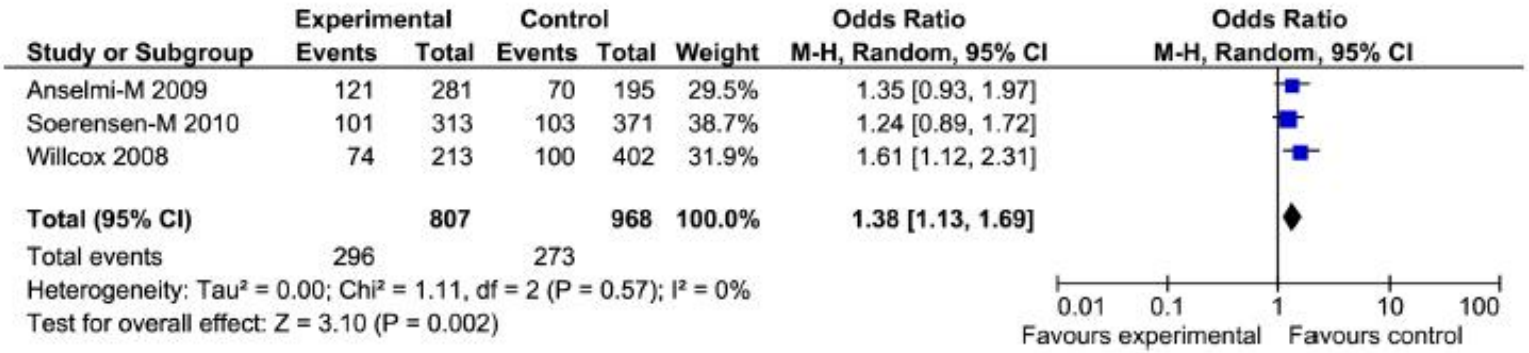



A




B

**Fig. (3). Meta-analysis of three case-control studies of the FOXO3A rs2764264 polymorphism and longevity using the random-effects model.** The odds ratio and 95% confidence interval (CI) for the effect of the C allele on longevity for the whole population (3A) and for males only (3B) are plotted on the two graphs. Studies are arranged chronologically based on the year of publication. M-H: Manten-Hanzel; C-I: Confidence Interval.

674 *Current Vascular Pharmacology*, 2014, *12*, 674-681

## Association between Genetic Variations in the Insulin/Insulin-Like Growth Factor (Igf-1) Signaling Pathway and Longevity: A Systematic Review and Meta-Analysis

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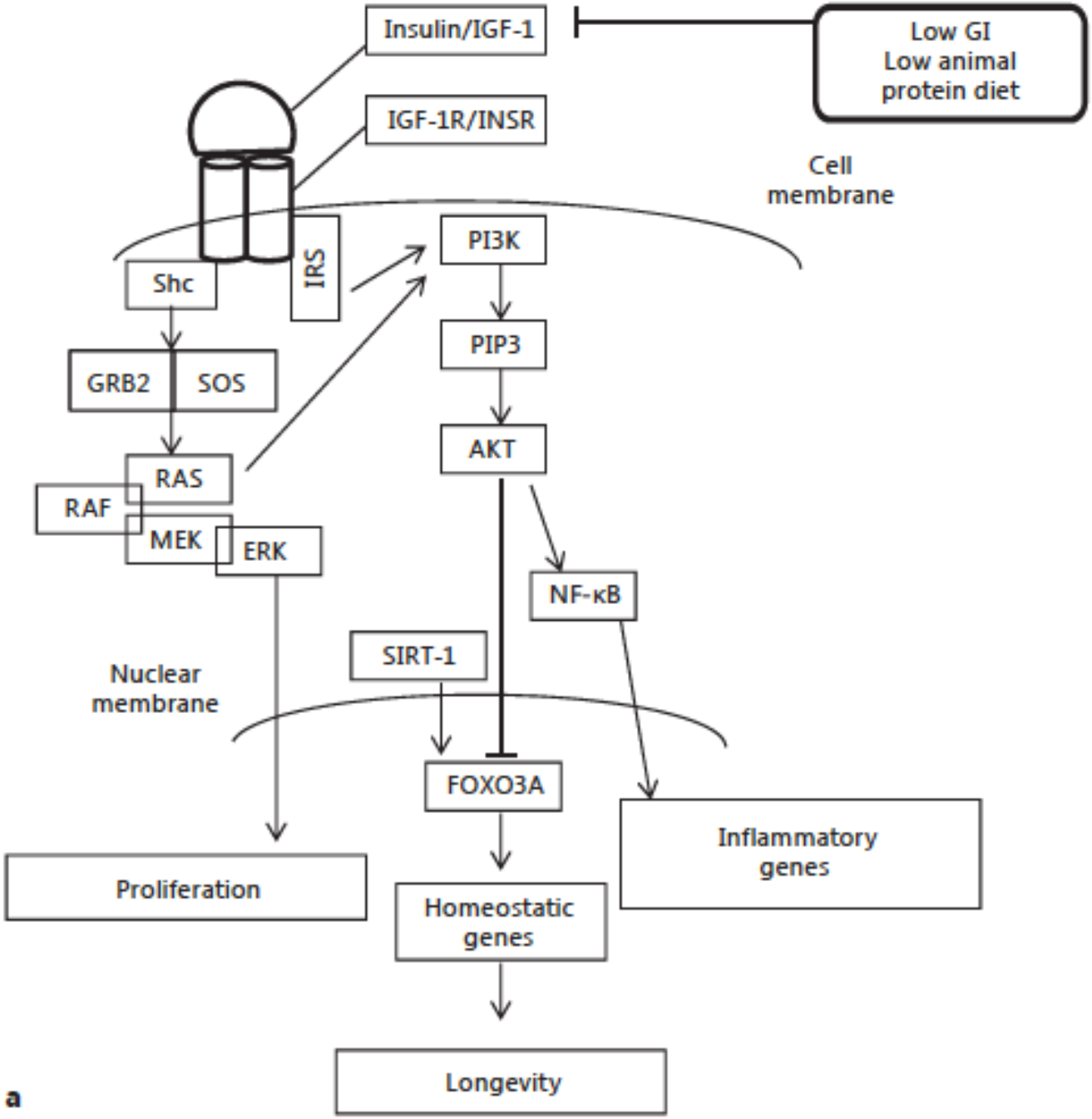
**Fig. 2.** Nutrient-sensing pathways modulate lifespan in humans by interacting with the environment. **a** Insulin/IGF-1 signalling pathway. Insulin (or IGF-1) binds its receptor (IRS) and interacts with PI3K. PI3K activates the second messenger PIP3. PIP3 messenger leads to the activation of AKT that inhibits FOXO3A, preventing the transcription of homeostatic genes. Also, SIRT1 can act on FOXO3A. Moreover, the insulin/IGF-1 signalling determines the activation of the RAS pathway leading to its mitogenic effect. In addition, AKT activates the NF-kB pathway allowing the transcription of inflammatory genes. MedDiet with low GI and/or with low protein intake may reduce the IGF-1 levels and may down-regulate the insulin/IGF-1 pathway leading to the transcription of homeostatic genes and stopping the mitogenic effect of RAS. This favours survival and longevity.




Clinical Section / Viewpoint

Gerontology

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**Mediterranean Diet and Healthy Ageing: A Sicilian Perspective**

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Giulia Accardi[d] Calogero Caruso[d]

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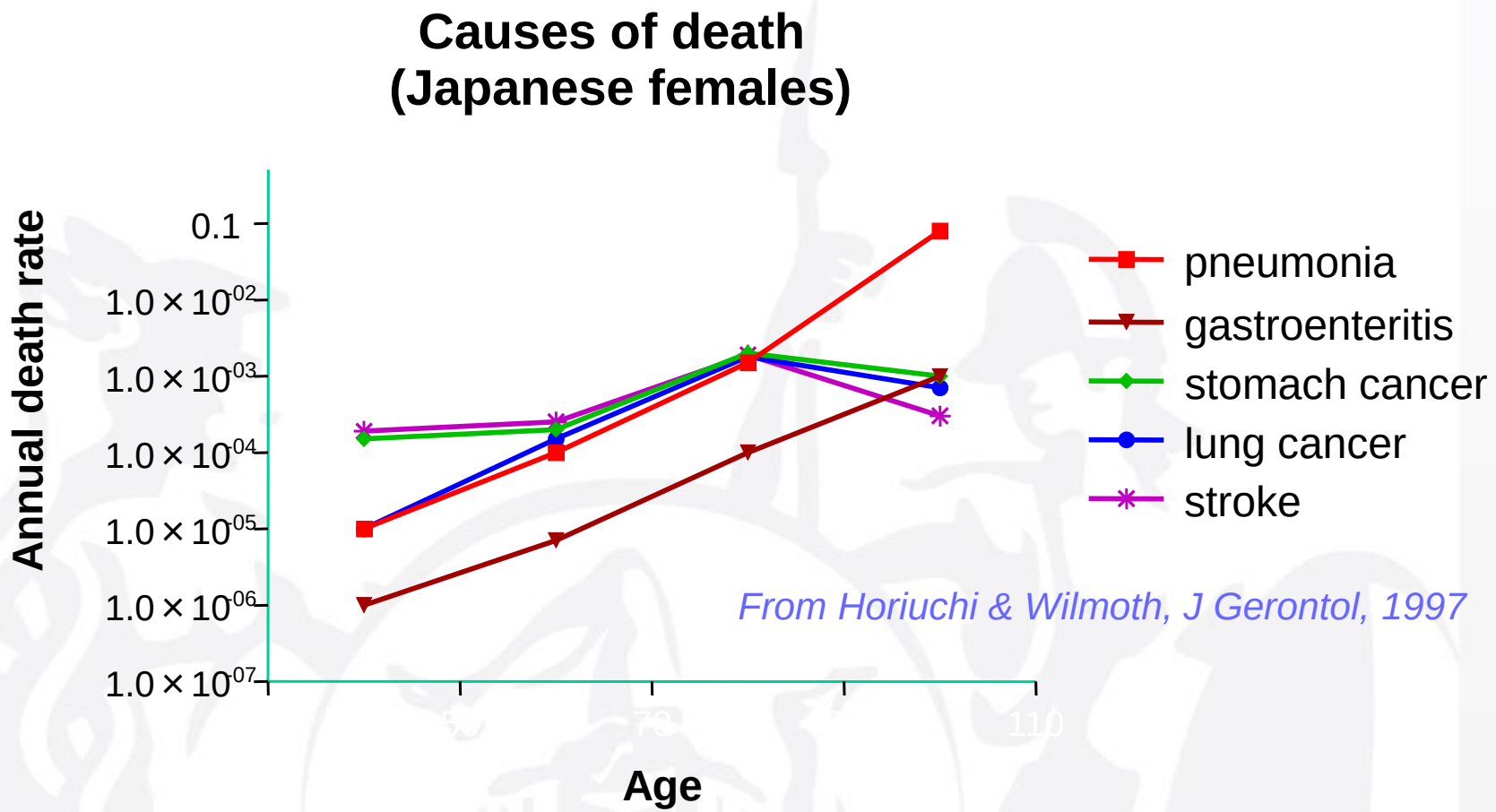
# Rate of death from infectious disease continues to accelerate with age




*From Horiuchi & Wilmoth, J Gerontol, 1997*

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# What is immunosenescence?

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**Immunity & Ageing**

BioMed Central

Editorial

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***Immunity & Ageing*: a new journal looking at ageing from an immunological point of view**

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## Immunity in ageing

In the elderly, many alterations in innate and clonotypic immunity have been described and viewed as deleterious, hence the term immunosenescence. In 1969, Roy Walford published his landmark book, "The Immunologic Theory of Aging", and first coined the term immunosenescence [6]. Significantly, most of the areas that he pioneered during his illustrious research career remain the "hot" areas of current gerontological research. On the other hand, immunosenescence is a complex process involving multiple reorganizational and developmentally regulated changes, rather than simple unidirectional decline of the whole function [7,8]. However, some immunological parameters are commonly notably reduced in the elderly and, reciprocally good function is tightly correlated to health status [4,5].

# What do we mean by "immunosenescence"?

Immunosenescence is a descriptive term for the deleterious age-associated changes to immunity observed in all mammals studied so far.

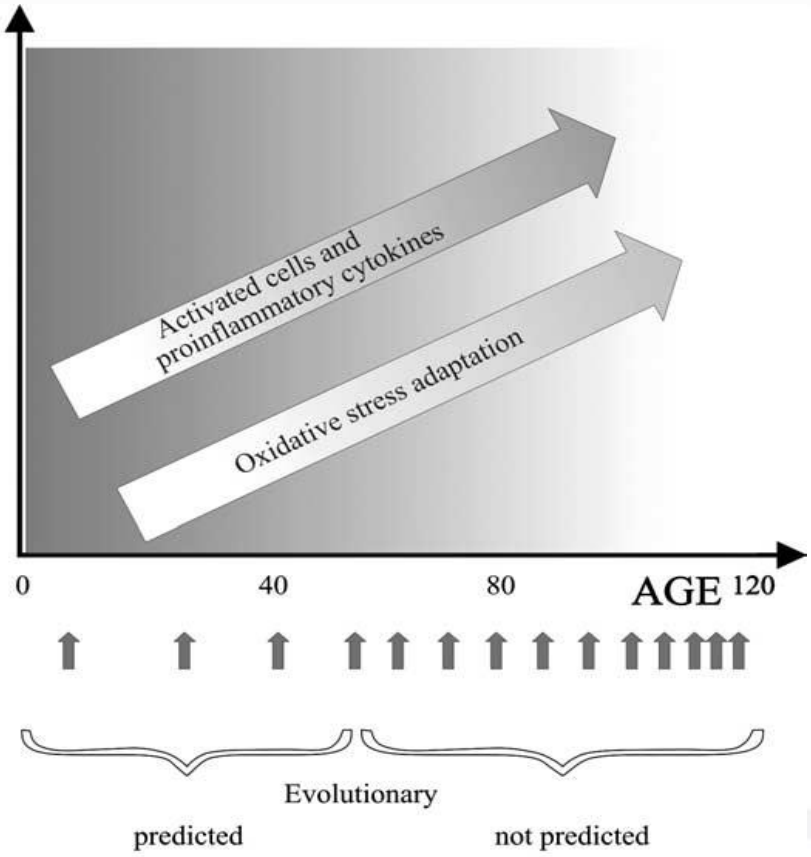
While all components of innate and instructive immunity are changed with age, the clinical impact of these changes is not clear, and mechanisms of and markers for immunosenescence are controversial

# The main age-associated changes reported in T cell immunity

**Table 1. The main age-associated changes reported in T cell immunity**

| Decreased | Increased |
|---|---|
| $CD3^+$ cells (slightly) | |
| T-cell receptor I (TCR1) (γδ) cells (slightly) | TCR1 and 2 oligoclonality |
| $CD4^+CD7^+$ cells | TCR variants (mutants) |
| $CD4^+$ cells (slightly or unchanged) | $CD4^+$ $CD8^+$ cells |
| $CD8^+$ cells (slightly or unchanged) | $CD3^+$ $CD25^+$ cells |
| $CD8^{+}$ regulatory cells | $CD3^{+}DR^{+}$ cells |
| $CD45RA^+$ cells | $CD45RO^+$ cells |
| $CD28^+$ cells | CD28-negative cells |
| Proliferation with mitogen | CD152 $(CTLA\text{-}4)^{+}$ cells |
| Interleukin receptor (IL2R) after activation | CD95-stimulated apoptosis, AICD |
| Cytotoxic T lymphocyte (CTL) generation | $CD95^+$ cells |
| CD40L (CD154) upregulation and B-cell help | $CD45RO^+CD60^+$ cells |
| bcl-2 | |
| TCR signal transduction | |
| p59fyn activity | |
| p56lck activity | |
| Nuclear transcription factor activation (AP-1, NF-AT, and NF-κB) | |
| IL 2 secretion | IL 10 secretion |
| Soluble IL 2R secretion | IL 6 secretion |
| IL 4 secretion | IL 4 secretion |
| Interferon-γ (IFN-γ) secretion | IFN-γ secretion |
| Telomere lengths | |
| DNA repair | DNA damage |

AICD: Activation – induced cell death; Parameters in red represent those for which a close approximation to consensus has been achieved.




LEGEND

= ag stimulation and ROS prodution

- **Antigenic load is associated with a loss of early memory cells, an increase of highly differentiated CD8+ cells, a gradual reduction of the immunological space**
- **As a consequence, a peculiar chronic inflammatory status characterizes immunosenescence.**

- **Lifelong chronic antigenic load induces age-related increase of activated immune cells and hyperproduction of proinflammatory cytokines.**

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M. De Martinis et al. FEBS Letters 579 (2005) 2035–2039

**Inflammatory responses are**

**physiologically crucial for survival**

**and constitute an essential part of our robustness**

**but …at the same time inflammation**

**is a basic component of frailty**

**and of most major age-related pathologies**

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Mechanisms of Ageing and Development 128 (2007) 83–91

Mechanisms of Ageing and Development

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# Inflammatory networks in ageing, age-related diseases and longevity

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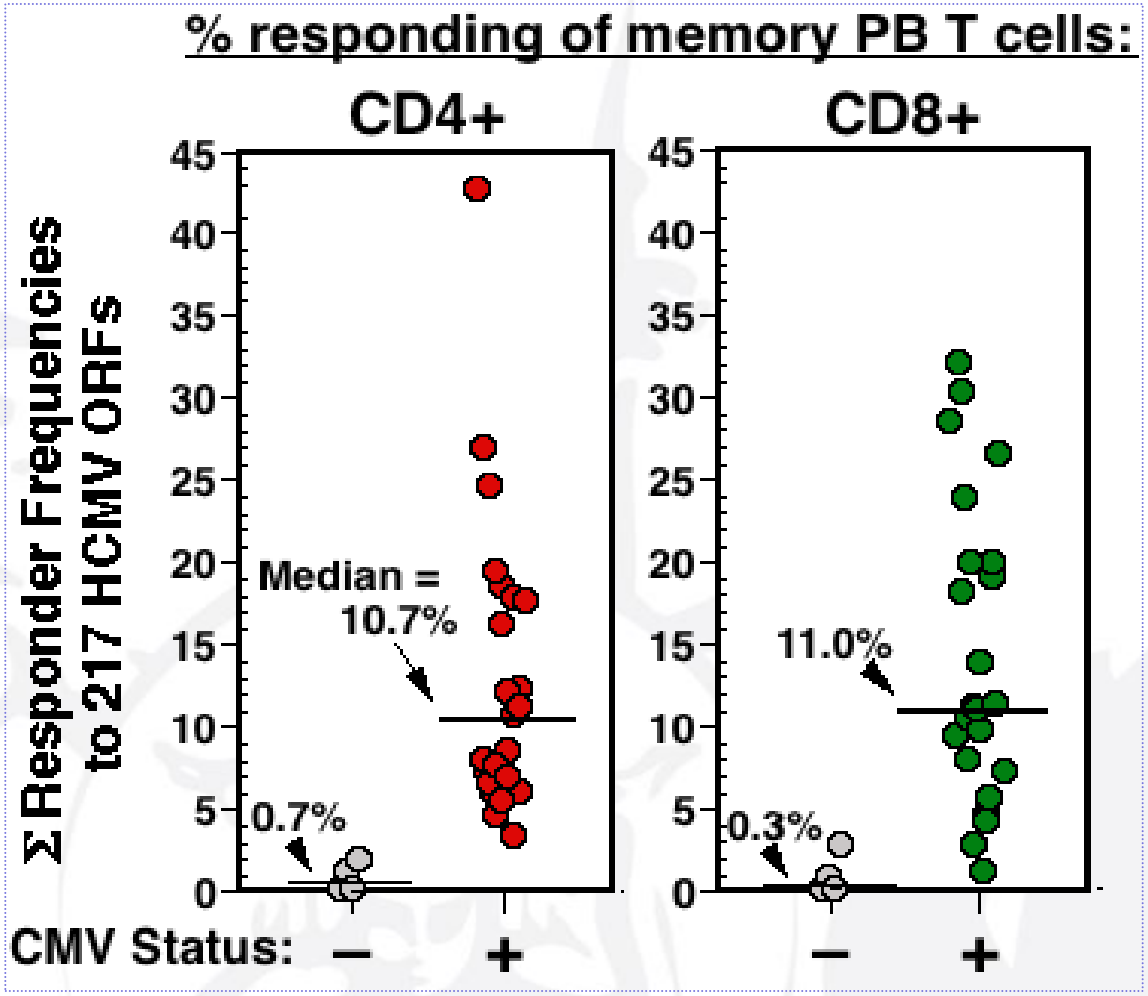
Available online 21 November 2006

*CMV has received scrutiny as a possible driver of immune senescence because the CD4+ and CD8+ T cell responses to this persistent virus can be enormous:*

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**Often > 10% of the memory repertoire!**

Graham Pawelec
Arne Akbar
Calogero Caruso
Beatrix Grubeck-Loebenstein
Rafael Solana
Anders Wikby

# Human immunosenescence: is it infectious?

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*Vol. 205: 257–268*
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0105-2896

Summary: Morbidity and mortality due to infectious disease is greater in the elderly than in the young, at least partly because of age-associated decreased immune competence, which renders individuals more susceptible to pathogens. This susceptibility is particularly evident for novel infectious agents such as in severe acute respiratory syndrome but is also all too apparent for common pathogens such as influenza. Many years ago, it was noted that the elderly possessed oligoclonal expansions of T cells, especially of $CD8^+$ cells. At the same time, it was established that cytomegalovirus (CMV) seropositivity was associated with many of the same phenotypic and functional alterations to T-cell immunity that were being reported as biomarkers associated with aging. It was discovered that CMV was the prime driving force behind most of the oligoclonal expansions and altered phenotypes and functions of CD8 cells. Independently, longitudinal studies of a free-living population of the very old in Sweden over the past decade have led to the emerging concept of an 'immune risk phenotype' (IRP), predicting mortality, which was itself found to be associated with CMV seropositivity. These findings support our hypothesis that the manner in which CMV and the host immune system interact is critical in determining the IRP and hence is predictive of mortality. In this sense, then, we suggest that immunosenescence is contagious.

## Immunity and aging

### Study motivation and problems of immunogerontological investigations in humans

For more than a quarter century, immunologists have collected data on immune parameters in the elderly in order to establish reliable 'biomarkers of aging' in the immune system for use in monitoring perceived deleterious alterations in immunity in old people and eventually for use in developing safe and effective recuperative interventions. Over the years, many differences between immune parameters in the young and old have been documented. However, the literature is full of confusing and conflicting data, which in the past has resulted in many immunologists dismissing the study of immunity and aging in humans as immature and unreliable. In *Table 1*, we list some of the most thoroughly investigated parameters focusing on T-cell immunity, the subject of the present review.



Opinion TRENDS in Immunology Vol. 25 No. 8 August 2004

# Is immunosenescence infectious?

**Graham Pawelec[1], Arne Akbar[2], Calogero Caruso[3], Rita Effros[4], Beatrix Grubeck-Loebenstein[5] and Anders Wikby[6]**

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**Herpes viruses are endemic. Once established, the virus is never eliminated but persists throughout life. The fraction of infected individuals gradually increases with age, such that the majority of elderly people are cytomegalovirus (CMV)$^+$, Epstein–Barr virus (EBV)$^+$ and Varicella$^+$. Clinically relevant reactivation of Varicella causes painful shingles; CMV reactivation can cause fatal pneumonia. Overt reactivation, even in the very elderly, occurs only in immunocompromised individuals; however, the necessity for maintaining immunity to these viruses is costly. We argue that this cost is not only reflected in the requirement for continuous immunosurveillance against these viruses but, more importantly, results in a re-configuration of T-cell immunity due to the accumulation of dysfunctional virus-specific cells, which fail to be eliminated from the system. Thus, we hypothesize that it is the chronic antigenic stimulation by CMV (and possibly other persisting antigens) that leads to an increasing prevalence of senescent, dysfunctional T cells, and therefore contributes to more general alterations in the immune system, which are associated with earlier mortality.**

Maintenance of protective immunity against cytomegalovirus (CMV) is clearly essential, which is graphically illustrated by earlier experiences in bone marrow transplantation (reviewed in Ref. [1]). Nonetheless, early reports of viral reactivation suggest that immunity is a continuous battle even in normal healthy persons [2]. At that time, the composition of the different T-cell subsets was just being identified, and, concurrently, reports were appearing that CMV infection could markedly alter components of these subsets [3]. One of the early studies showed an increased number of $CD8^+$ cells in normal healthy donors and even identified expansions of $CD8^+CD57^+$ (HNK-1$^+$) subsets in CMV seropositive individuals. It was over a decade later that differences between young and old donors were assessed in the context of CMV status [4]. That study reported that CMV seropositivity was associated with an increased number of both $CD4^+$ and $CD8^+$ cells, which were $CD28^-$. Importantly, the authors pointed out that this phenotype had previously been associated with age; however, they found that it was primarily associated with CMV status and only secondarily with age, given the increasing frequency of CMV-infection with age. However, both age and CMV status influenced the number of $CD8^+$ cells and their expression of CD45RA and CD28.

**Role of CMV in determining the 'immune risk phenotype'**
Age-associated changes in the immune system have been extensively documented over the years, without reference to CMV status (Box 1). However, two different approaches have recently begun to shed more light on the unexpected way in which CMV infection shapes the ageing human immune system. The first is the development of tetramer technology, enabling direct identification of T cells carrying receptors for single peptide epitopes. The second is the

**Box 1. Alterations in the T-cell compartment with age**

- ↑ $CD45RO^+$ cells (reviewed in Ref. [41])
- ↓ $CD45^+$ cells (reviewed in Ref. [41])
- ↓ CD28 expression (reviewed in Ref. [41])
- ↑ CD152 expression (reviewed in Ref. [41])
- ↑ killer cell lectin-like receptor G1 (KLRG-1) expression [15]
- ↓ apoptosis of CD8 cells (reviewed in Ref. [41])
- ↑ apoptosis of CD4 cells (reviewed in Ref. [41])
- ↓ interferon-γ (IFN-γ) production; meta-analysis [42]*
- ? interleukin-2 (IL-2) production; meta-analysis [42]*
- ↓ telomere lengths (reviewed in Ref. [41])
- ↓ telomerase induction (reviewed in Ref. [41])
- ↑ DNA damage (reviewed in Ref. [41])
- ↓ DNA repair (reviewed in Ref. [41])
- ↓ stress resistance and heat-shock protein (HSP) expression (reviewed in Ref. [41])

*Data from meta-analysis of 23 studies, 11 reported decreased IFN-γ production, 8 no change and 4 an increase.
[b]Data from meta-analysis of 28 studies, 14 reported decreased IL-2 production, 3 no change and 11 an increase.

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# What is CMV?

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# CMV Infection & disease

- ***Latent CMV infection*** represents lifelong persistence of virus without replication in healthy seropositive host.
- ***CMV active infection*** : actively replicating virus, can be diagnosed by nucleic acid-based assays or antigenemia.
- ***CMV disease*** is defined by evidence of CMV infection with attributable symptoms, it can be :-
  - ***CMV syndrome*** (fever, fatigue, leukopenia and/or thrombocytopenia, and an increased CMV titer from a specific diagnostic assay)
  - ***Invasive CMV disease*** (e.g., pneumonitis, hepatitis, or gastrointestinal involvement such as colitis or enteritis, or involvement of the allograft itself).

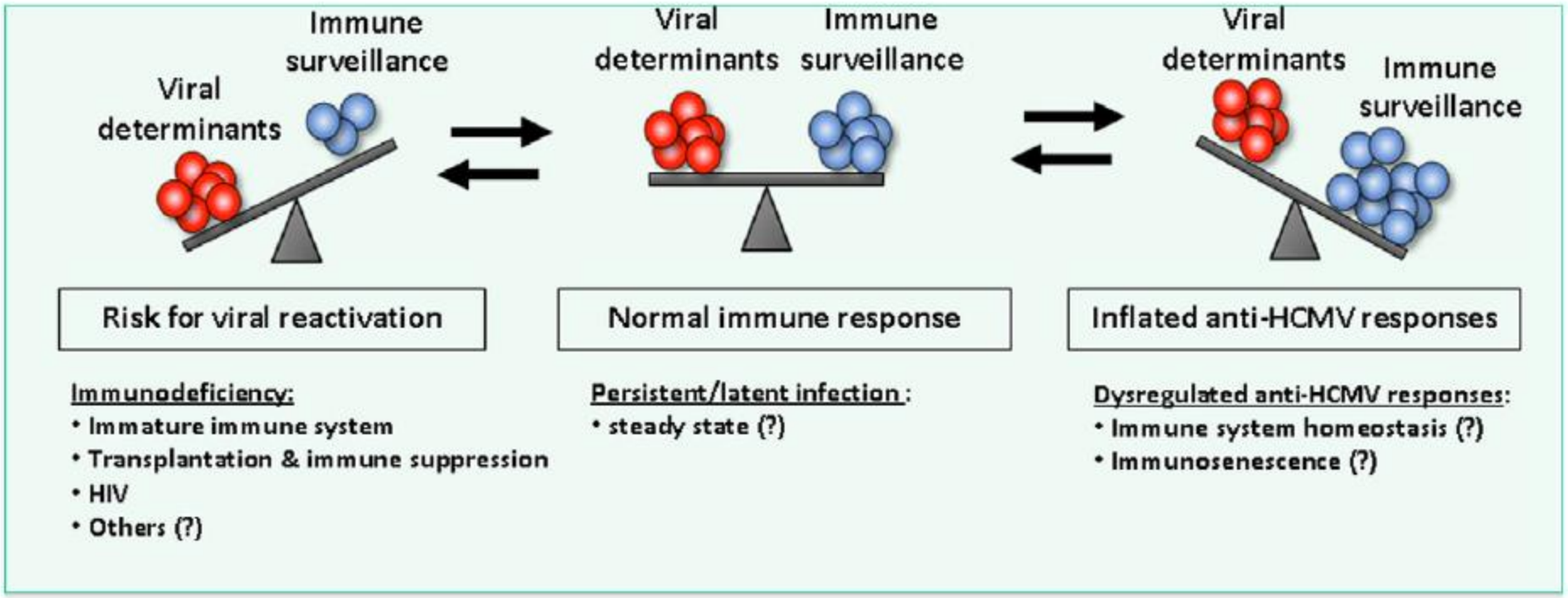


**Fig. 1.** Host:HCMV interaction.

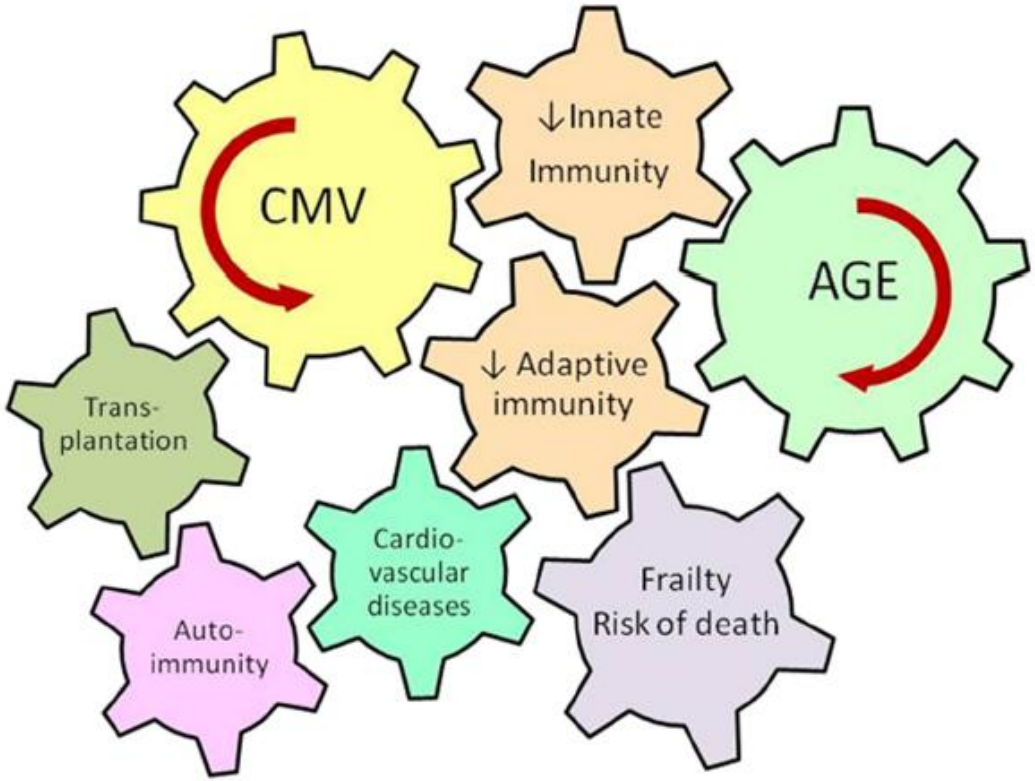


**Figure 2 Age and CMV infection are major driving forces contributing to the deterioration of innate and adaptive immunity.** Age-associated decrease of adaptive immunity is termed immunosenescence. The deregulation of innate immunity is associated with inflammageing. Immunosenescence and inflammageing play a significant role in the pathogenesis of different clinical situations that can lead to increased risk of frailty and death in the elderly.

# What about genetic control?

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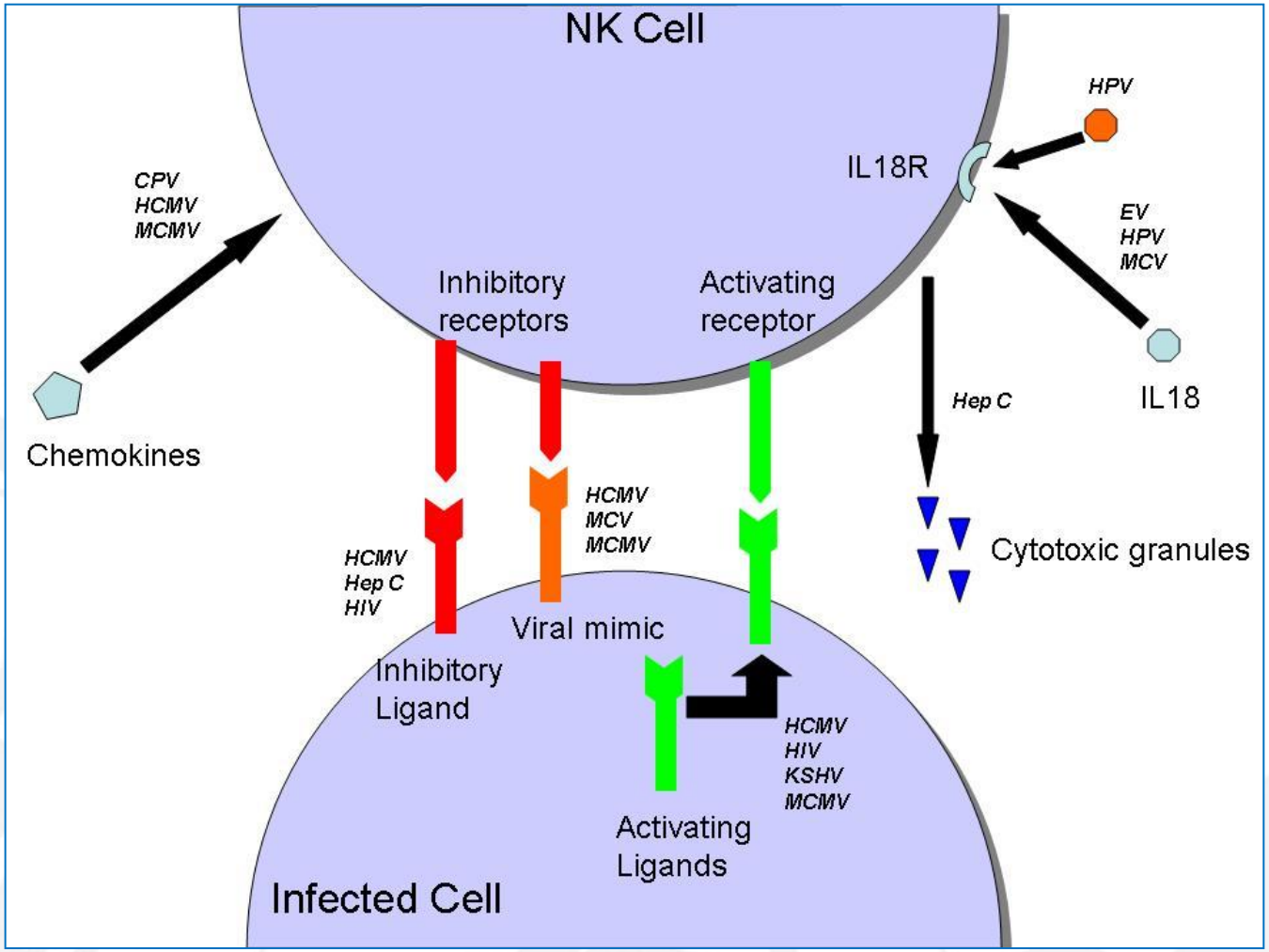
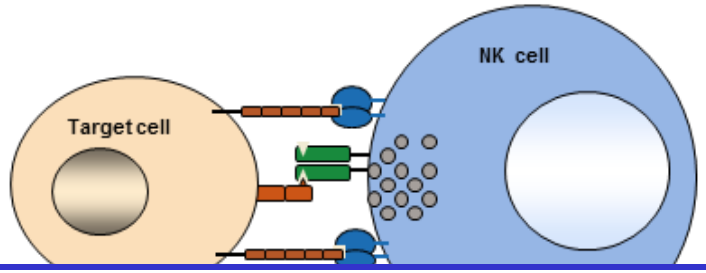
# NK cell


NK Cell
HPV
IL18R
CPV
HCMV
MCMV
EV
HPV
MCV
Inhibitory
receptors
Activating
receptor
Hep C
IL18
Chemokines
HCMV
MCV
MCMV
HCMV
Hep C
HIV
Cytotoxic granules
Viral mimic
Inhibitory
Ligand
HCMV
HIV
KSHV
MCMV
Activating
Ligands
Infected Cell

# KIR: biological function, genetic characterization and protein structure

## ❑ Biological function

- Expressed on NK cells and on some T lymphocytes (CD8)
- Regulation of NK cells through interaction with **HLA class I ligands**




**Peripheral circulating lymphocytes and key protagonists of innate immune responses**

**Association between KIR/KIR ligands and diseases**

*Review Article*

**KIR/HLA Interactions and Pathogen Immunity**

Khaleel M. Jamil and Salim I. Khakoo

The innate immune system is the first line of defence in response to pathogen infection. Natural killer (NK) cells perform a vital role in this response with the ability to directly kill infected cells, produce cytokines, and cross-talk with the adaptive immune system. These effector functions are dependent on activation of NK cells which is determined by surface receptor interactions with ligands on target cells. Of these receptors, the polymorphic killer immunoglobulin-like receptors (KIRs), which interact with MHC class 1 (also highly polymorphic), are largely inhibitory, and exhibit substantial genetic diversity. The result is a significant variation of NK cell repertoire between individuals and also between populations, with a multitude of possible KIR:HLA combinations. As each KIR:ligand interaction may have differential effects on NK cell activation and inhibition, this diversity has important potential influences on the host response to infections. Genetic studies have demonstrated associations between specific KIR:ligand combinations and the outcome of viral (and other) infections, in particular hepatitis C and HIV infection. Detailed functional studies are not required to define the mechanisms underpinning these disease associations.

Killer cell immunoglobulin-like receptor gene associations with autoimmune and allergic diseases, recurrent spontaneous abortion, and neoplasms

Piotr Kusnierczyk *

Killer cell immunoglobulin-like receptors (KIRs) are a family of cell surface inhibitory or activating receptors expressed on natural killer cells and some subpopulations of T lymphocytes. *KIR* genes are clustered in the 19q13.4 region and are characterized by both allelic (high numbers of variants) and haplotypic (different numbers of genes for inhibitory and activating receptors on individual chromosomes) polymorphism. This contributes to diverse susceptibility to diseases and other clinical situations. Associations of *KIR* genes, as well as of genes for their ligands, with selected diseases such as psoriasis vulgaris and atopic dermatitis, rheumatoid arthritis, recurrent spontaneous abortion, and non-small cell lung cancer are discussed in the context of NK and T cell functions.

Keywords: KIR genes, skin diseases, rheumatoid arthritis, spontaneous abortion, cancer, viral diseases, viral infections

The Yin and Yang of *HLA* and *KIR* in human disease

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ABSTRACT

Killer cell immunoglobulin-like receptors (KIR) are expressed on natural killer (NK) cells and subsets of T cells. The *KIR* genes are polymorphic and the *KIR* gene complex is polygenic with varying numbers of inhibitory and activating receptors. HLA class I molecules serve as ligands for the KIR. Interactions of the independently segregating *KIR* and *HLA* loci are important for recognition of targets by NK cells as well as NK cell 'licensing'. Several disease association studies indicate a role for interactions between these loci in infectious diseases, autoimmune/inflammatory disorders, cancer and reproduction. Emerging functional data supports a mechanism based on a continuum of inhibition to activation through various compound *KIR–HLA* genotypes in diseases.



- Infection diseases (HCV, HBV, HIV, CMV, ...)
- Autoimmune disorders (psoriasis, rheumatoid arthritis, ...)
- Cancer
- Pregnancy-related
- ...

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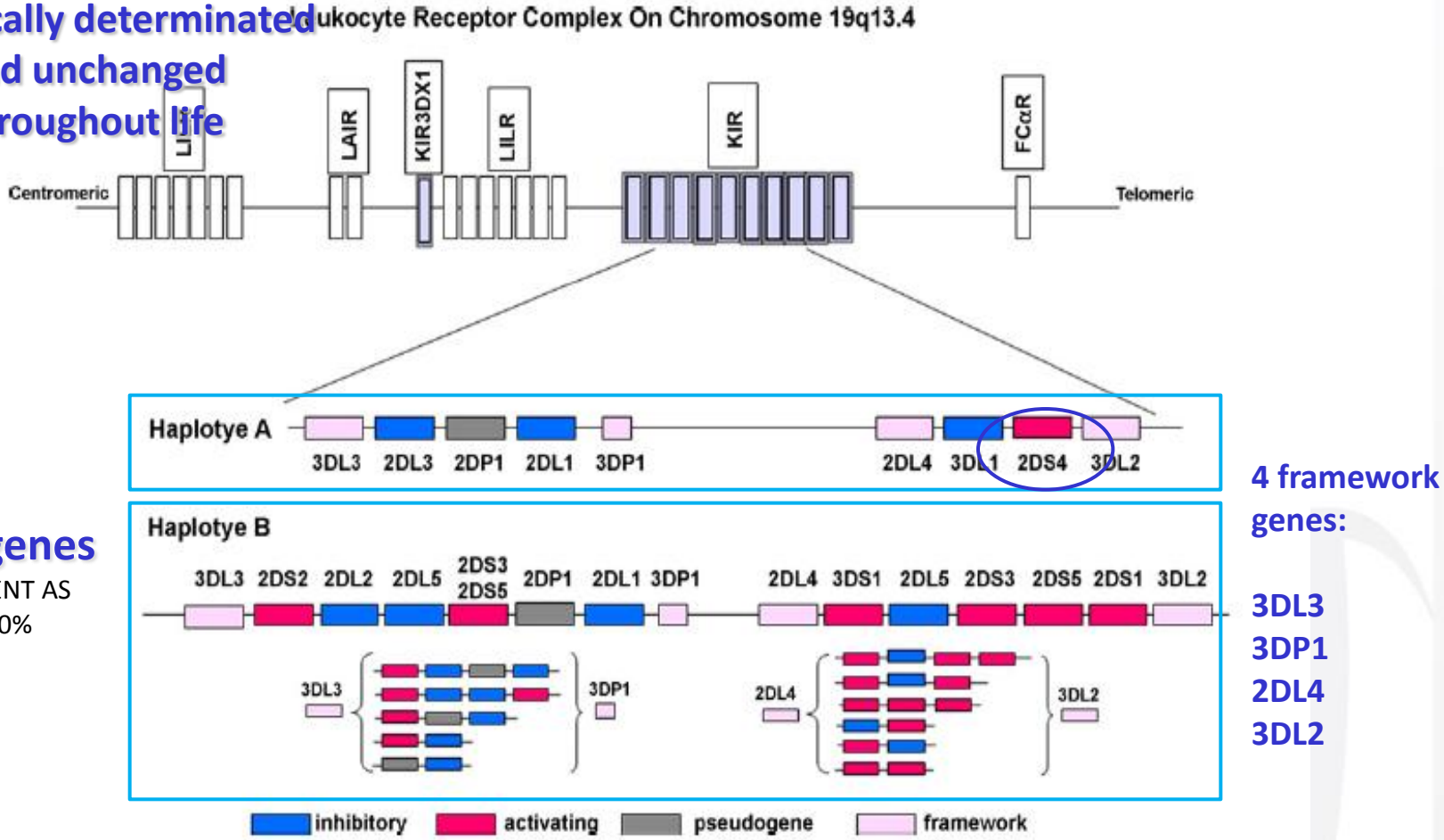
# KIR: biological function, genetic organization and protein structure

## ❑ Genetic organization

**Kir gene family**

**Genetically determinated and unchanged throughout life**




**15 loci**

**2 pseudogenes**

KIR2DS4 IS PRESENT AS NULL ALLELE in 80% OF CASES

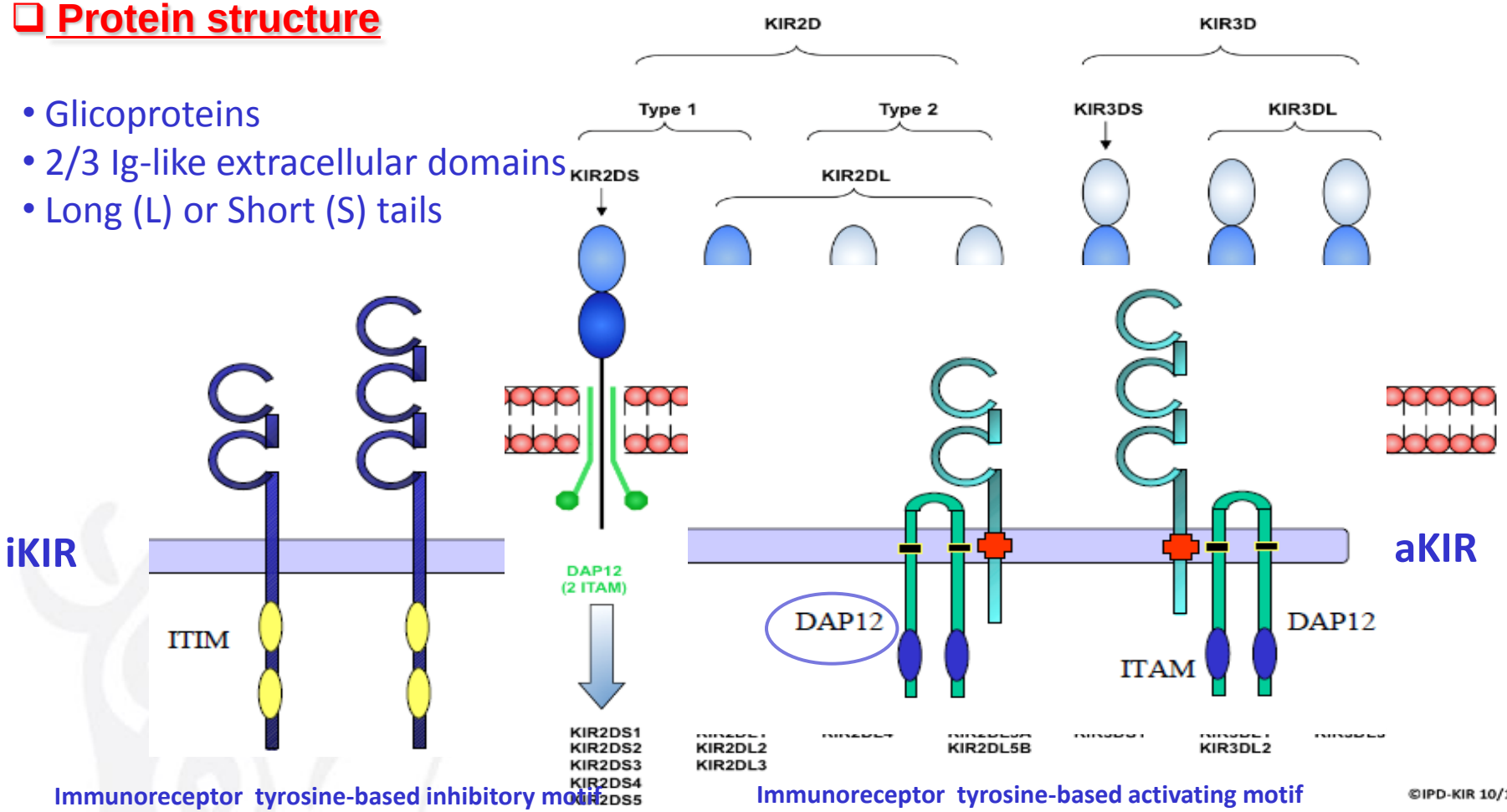
**4 framework genes:**

**3DL3**
**3DP1**
**2DL4**
**3DL2**

# KIR: biological function, genetic organization and protein structure

## ❑ Protein structure

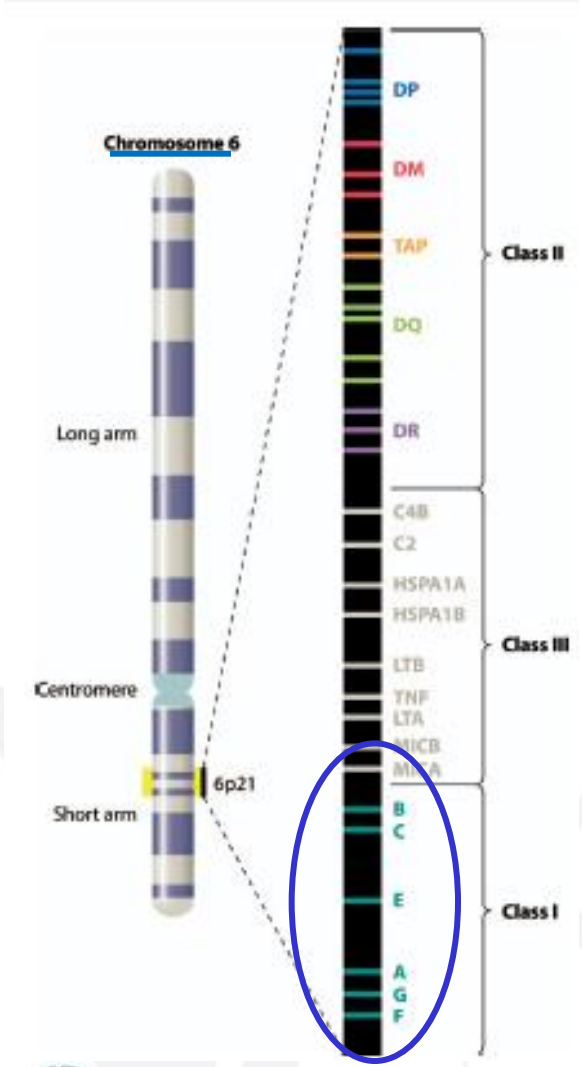
- Glicoproteins
- 2/3 Ig-like extracellular domains
- Long (L) or Short (S) tails




**Both signals exist but NK cells regulation dipends on the combination of these effects**

**Inhibitory signal** **Activating signal**

# KIR HLA ligands and interactions




- **Extremely polymorphic**
- **Different function**
- **Varying susceptibility to pathogens and diseases**

| Activating KIR | Ligand |
|---|---|
| 2DS1 | HLA-C2 (weaker than 2DL1) |
| 2DS2 | HLA-C1 (weak), HLA-A*11:01 |
| 2DS3 | Unknown |
| 2DS4 | HLA-C*05:01, A*11:02, C*16:01 |
| 2DS5 | Unknown |
| 3DS1 | Unknown |
| **Inhibitory KIR** | **Ligand** |
| 2DL1 | HLA-C2 (N77/K80) |
| 2DL2 | HLA-C1 (S77/N80), HLA-C2, HLA-B*46:01 and HLA-B*73:01 (C1 epitope) |
| 2DL3 | HLA-C1 (S77/N80), HLA-C2, HLA-B*46:01 and HLA-B*73:01 (C1 epitope) |
| 2DL4 | HLA-G (intracellular interaction?) |
| 2DL5A/B | Unknown |
| 3DL1 | HLA-A (with Bw4 motif), HLA-Bw4 |
| 3DL2 | HLA-A3/A11 |
| 3DL3 | Unknown |

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The lack of specific KIR-HLA receptor-ligand pairing leads to functionally null phenotypes

# The importance of interactions between KIR/KIR ligands and diseases

## Support vector machine algorithms in the search of KIR gene associations with disease

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ABSTRACT

Killer-cell immunoglobulin-like receptors (KIR) are membrane proteins expressed by natural killer cells and CD8 lymphocytes. The KIR system consists of 17 genes and 614 alleles, some of which bind human leukocyte antigens (HLA). Both KIR and HLA modulate susceptibility to haematological malignancies, viral infections and autoimmune diseases. Molecular epidemiology studies employ traditional statistical methods to identify links between KIR genes and disease. Here we describe our results at applying artificial intelligence algorithms (support vector machines) to identify associations between KIR genes and disease. We demonstrate that these algorithms are capable of classifying samples into healthy and diseased groups based solely on KIR genotype with potential use in clinical decision support systems.



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# KIR/KIR ligands disease associations

KIR–HLA disease associations

| Disease | KIR–HLA ligand pair | Effect | Reference |
|---|---|---|---|
| Infectious diseases | | | |
| HIV | *KIR3DS1/Bw4-80I* | Slower progression | [40] |
| | *KIR3DL1*004/Bw4* | Slower progression | [69] |
| | *KIR3DL1*h/Bw4 80I* | | |
| | *KIR3DS1* | Reduced risk of infection | [64,65,67] |
| HCV | *KIR2DL3/HLA-C1* homozygosity | Resolution of infection | [71] |
| Human cytomegalovirus (HCMV) | KIR2DL1 expression on all NK cells | Recurrent CMV infection | [75] |
| | ≥1 activating *KIR* in donor in bone marrow transplantation | Protection from CMV reactivation in the recipient | [76] |
| Herpes simplex virus (HSV) | *KIR3DS1* in absence of *Bw4* | Reactivation of HSV during IRD in HIV | [77] |
| *M. tuberculosis* | *KIR2DL1*; *KIR2DL3* | Susceptibility | [78] |
| *P. falciparum* | *KIR3DL2*002* | High response to infected RBCs | [82] |
| Autoimmune and inflammatory conditions | | | |
| Psoriatic arthritis | *KIR2DS1/2DS2*; *HLA-Cw* group homozygosity | Susceptibility | [94,125] |
| Psoriasis | *KIR2DS1/HLA-Cw06* | Susceptibility | [92] |
| | *KIR2DS1*; *KIR2DL5*; *KIR* haplotype B | Susceptibility | [91] |
| Rhuematoid vasculitis | *KIR2DS2/HLA-Cw03* | Susceptibility | [87] |
| Scleroderma | *KIR2DS2+/2DL2−* | Susceptibility | [90] |
| Acute coronary syndrome | De novo expression of KIR2DS2 on CD4+CD28$^{null}$cells | Susceptibility | [88] |
| IDDM | *KIR2DS2/HLA-C1* | Susceptibility | [99] |
| Endometriosis | *KIR3DS1/Bw4* | Protection | [95] |
| Birdshort chorioretinopathy | Weak inhibitory *KIR/HLA* combinations and activating *KIR* in HLA-A*29+ individuals | Susceptibility | [96] |
| Idiopathic bronchiectasis | *HLA-C1/C1 and 2DS1/2DS2* | Susceptibility | [97] |
| Primary sclerosing cholangitis | *KIR3DL1/Bw4*; *KIR2DL1/HLA-C2* | Protection | [98] |
| Cancer | | | |
| Malignant melanoma | *KIR/2DL2/2DL3*; *HLA-C1* | Susceptibility | [101] |
| Leukemia | *KIR2DL1*; *KIR2DL2*; *KIR2DL3* | Susceptibility | [103] |
| Hodgkin's lymphoma | *KIR2DS1*; *KIR3DS1* | Protection | [106] |
| Nasopharyngeal carcinoma | ≥5 activating *KIR* | Susceptibility | [108] |
| Cervical cancer | *KIR3DS1*and absence of *HLA-C2* and/or *HLA-Bw4* | Susceptibility | [107] |
| T-LGL | Expression of inhibitory KIR in absence of ligands | More severe disease | [112] |
| NK-LGL | Expression of activating KIR | May contribute to disease pathogenesis | [111,113] |
| Sezary syndrome | Expression of KIR3DL2 | Useful diagnostic marker | [110] |
| Reproduction | | | |
| Preeclampsia | Mothers with AA *KIR* genotype; fetus with *HLA-C2* | Susceptibility | [117] |
| Recurrent miscarriages/spontaneous abortions | Lack of *KIR2DS1* in mothers and increased frequency of *HLA-C2* in both mother and male partner | Susceptibility | [120] |
| | Increased *KIR2DS2* and decreased *HLA-C2* frequency, overall increased frequency of activating *KIR* | Susceptibility | [121] |
| | Higher cell surface expression of KIR2DL4 | Susceptibility | [122] |

**Depending on the number of Ig domains 2/3, they are named with 2D or 3D suffix**

**Long (L) or Short (S) instead indicate the long or short intracytoplasmic domain, respectively inhibitory or activatory**

**The last number identifies the gene**

**HLA-C1, C alleles with asparagine at position 80**

**HLA-C2, C alleles with lysine at position 80**

**HLA-Bw4-I, Bw4 alleles with isoleucine at position 80**

**HLA-Bw4-T, Bw4 alleles with threonine at position 80**

# HLA and Killer Cell Immunoglobulin-like Receptors Influence the Natural Course of CMV Infection

**Danilo Di Bona,[1,2] Valeria Scafidi,[3] Antonella Plaia,[4] Claudia Colomba,[5] Domenico Nuzzo,[3] Cecilia Occhino,[6] Antonino Tuttolomondo,[7] Giovanni Giammanco,[5] Simona De Grazia,[5] Giuseppe Montalto,[7] Giovanni Duro,[3] Marco Cippitelli,[8] and Calogero Caruso[1,2]**

***Background.*** Natural killer (NK) cells provide a major defense against cytomegalovirus (CMV) infection through the interaction of their surface receptors, including the activating and inhibitory killer immunoglobulin-like receptors (KIRs), and human leukocyte antigens (HLA) class I molecules. This study assessed whether the KIR and HLA repertoire may influence the risk of developing symptomatic or asymptomatic disease after primary CMV infection in the immunocompetent host.

***Methods.*** Sixty immunocompetent patients with primary symptomatic CMV infection were genotyped for KIR and their HLA ligands, along with 60 subjects with a previous asymptomatic infection as controls.

***Results.*** The frequency of the homozygous A haplotype (only KIR2DS4 as activating KIR) was higher in symptomatic patients than controls (30% vs 12%, respectively; odds ratio [OR] = 3.24; $P = .01$). By logistic regression, the risk of developing symptomatic disease was associated with the homozygous A haplotype and the HLABw4$^{T}$ allele. Combining the 2 independent variables, we found that 37 out of 60 (62%) symptomatic patients but only 18 out of 60 (30%) of controls possessed the homozygous A haplotype or the HLABw4$^{T}$ allele with a highly significant OR (OR = 3.75, $P < .0005$).

***Conclusions.*** Immunocompetent subjects carrying the homozygous A haplotype or the HLABw4$^{T}$ allele are at higher risk of developing symptomatic disease after primary CMV infection.

**Table 1. Frequencies of KIR, HLA, and KIR-HLA Combinations Among Individuals With Symptomatic and Asymptomatic CMV Infection**

| Genetic Factor | Frequency Symptomatic Infection N = 60 N (%) | Frequency Asymptomatic Infection N = 60 N (%) | OR | *P* Value |
|---|---|---|---|---|
| Haplotype AA | 18 (30) | 7 (12) | 3.24 | .01[a] |
| Haplotype AB + BB | 42 (70) | 53 (88) | | |
| 2DS2 | 16 (27) | 27 (45) | 0.44 | .03[a] |
| HLA-C1 | 23 (38) | 25 (42) | 0.87 | .7 |
| HLA-C2 | 44 (73) | 36 (60) | 1.83 | .1 |
| HLA-C1C1 | 10 (17) | 8 (13) | 1.30 | .6 |
| HLA-C1C2 | 13 (22) | 17 (28) | 0.7 | .4 |
| HLA-C2C2 | 31 (52) | 19 (32) | 2.31 | .03[a] |
| 2DL2 + HLA-C1 | 12 (20) | 17 (28) | 0.63 | .28 |
| 2DL3 + HLA-C1 | 18 (30) | 18 (30) | 1.00 | 1.00 |
| 2DS2 + HLA-C1 | 4 (7) | 13 (27) | 0.26 | .02[a] |
| 2DL2 + HLA-C1C1 | 5 (8) | 6 (10) | 0.98 | .98 |
| 2DL3 + HLA-C1C1 | 7 (12) | 6 (10) | 1.19 | .76 |
| 2DS2 + HLA-C1C1 | 0 (0) | 4 (7) | . . . | . . . |
| 2DL1 + HLA-C2 | 41 (68) | 35 (58) | 1.54 | .25 |
| 2DS1 + HLA-C2 | 18 (30) | 19 (32) | 0.92 | .84 |
| 2DL1 + HLA-C2C2 | 28 (47) | 18 (30) | 2.04 | .06[b] |
| 2DS1 + HLA-C2C2 | 12 (20) | 9 (15) | 0.71 | .47 |
| HLA-Bw4$^{T}$ | 21 (35) | 12 (20) | 2.15 | .06[b] |
| HLA-Bw4$^{I}$ | 24 (40) | 22 (37) | 1.15 | .7 |
| HLA-Bw4$^{TT}$ | 17 (28) | 9 (15) | 2.24 | .07 |
| HLA-Bw4$^{TI}$ | 4 (7) | 3 (5) | 1.36 | .7 |
| HLA-Bw4$^{II}$ | 20 (33) | 19 (32) | 1.08 | .84 |
| 3DL1 + HLA-Bw4$^{T}$ | 19 (32) | 11 (18) | 2.06 | .09 |
| 3DL1 + HLA-Bw4$^{TT}$ | 16 (27) | 8 (13) | 2.36 | .07 |
| 3DL1 + HLA-Bw4$^{I}$ | 23 (38) | 21 (35) | 1.15 | .7 |
| 3DL1 + HLA-Bw4$^{II}$ | 20 (33) | 18 (30) | 1.17 | .7 |
| 3DS1 + HLA-Bw4$^{I}$ | 9 (15) | 12 (20) | 0.71 | .47 |
| 3DS1 + HLA-Bw4$^{II}$ | 6 (10) | 12 (20) | 0.44 | .1 |

*KIR2DS2* was the only KIR gene reported in the table because it was the only one differently expressed between cases and controls. The KIR-HLA interaction suggested by literature were analyzed and reported in the table.

Abbreviations: CMV, cytomegalovirus; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; OR, odds ratio.

[a] Statistical significance ($P < .05$).

[b] Marginal statistical significance ($P < .1$).

Individuals without activating receptors (AA haplotype) are more susceptible to develop symptomatic infection.

The activating KIR2DS2 gene is less frequent In the subjects with symptomatic infection than in controls.

The KIR2DS2-HLA-C1 phenotype is less frequent in the subjects with symptomatic Infection than in controls.

**Table 2. Logistic Regression Model to Predict the Occurrence of Symptomatic Infection in All Subjects (n = 120)**

| Variable | Code | β | SE | *P* Value | OR (95% CI) |
|---|---|---|---|---|---|
| Haplotype | 0: AB/BB Haplotypes | | | | |
| | 1: AA Haplotype | 1.64 | 0.56 | .003 | 5.14 (1.84–16.27) |
| HLA C1C2 | 1: HLA C2C2 | 1.62 | 0.61 | .008 | 5.03 (1.60–17.96) |
| | 2: HLA C1C2 | 0.85 | 0.65 | n.s | 2.34 (.68–8.87) |
| | 3: HLA C1C1 | 1.51 | 0.72 | .04 | 4.54 (1.14–20.11) |
| HLA-Bw4$^T$ | 0: Absent | | | | |
| | 1: Present | 0.96 | 0.47 | .04 | 2.62 (1.06–6.72) |
| (Conditioning variables) | | | | | |
| Sex | 0: female | | | | |
| | 1: male | . . . | . . . | n.s. | . . . |
| Age | 0: <36 y | | | | |
| | 1: ≥36 y | . . . | . . . | n.s. | . . . |

Sex and age did not enter into the final model after the stepwise procedure.

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; n.s., not significant; OR, odds ratio; SE, standard error.

Logistic regression confirms AA and demonstrates that HLA-Bw4-T is a predictor of the risk of symptomatic Infection.

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**Table 3. Frequencies of HLA and KIR-HLA Combinations Among AB/BB Haplotype Individuals With Symptomatic and Asymptomatic CMV Infection**

| Genetic Factor | Frequency Symptomatic Infection N = 42 *N* (%) | Frequency Asymptomatic Infection N = 53 *N* (%) | OR | *P* Value |
|---|---|---|---|---|
| HLA-C1 | 15 (35) | 23 (47) | 0.72 | .44 |
| HLA-C2 | 32 (76) | 33 (62) | 1.94 | .15 |
| HLA-C1C1 | 6 (14) | 8 (15) | 0.94 | .9 |
| HLA-C1C2 | 9 (21) | 15 (28) | 0.69 | .44 |
| HLA-C2C2 | 23 (55) | 18 (34) | 2.35 | .04[a] |
| 2DL2 + HLA-C1 | 12 (29) | 17 (32) | 0.85 | .71 |
| 2DL3 + HLA-C1 | 10 (24) | 16 (30) | 0.72 | .48 |
| 2DS2 + HLA-C1 | 4 (10) | 13 (25) | 0.32 | .06[b] |
| 2DL2 + HLA-C1C1 | 5 (12) | 6 (11) | 1.06 | .9 |
| 2DL3 + HLA-C1C1 | 3 (71) | 6 (11) | 0.60 | .49 |
| 2DS2 + HLA-C1C1 | 0 (0) | 4 (7) | . . . | . . . |
| 2DL1 + HLA-C2 | 30 (71) | 32 (60) | 1.64 | .26 |
| 2DS1 + HLA-C2 | 18 (43) | 19 (36) | 1.34 | .5 |
| 2DL1 + HLA-C2C2 | 21 (50) | 17 (32) | 2.12 | .07 |
| 2DS1 + HLA-C2C2 | 12 (29) | 9 (17) | 1.96 | .17 |
| HLA-Bw4$^{T}$ | 19 (45) | 11 (21) | 3.15 | .01[a] |
| HLA-Bw4$^{I}$ | 14 (33) | 20 (38) | 0.83 | .66 |
| HLA-Bw4$^{TT}$ | 15 (36) | 9 (17) | 2.72 | .03[a] |
| HLA-Bw4$^{TI}$ | 4 (9) | 2 (4) | 2.68 | .2 |
| HLA-Bw4$^{II}$ | 10 (24) | 18 (34) | 0.61 | .2 |
| 3DL1 + HLA-Bw4$^{T}$ | 17 (40) | 10 (19) | 2.92 | .02[a] |
| 3DL1 + HLA-Bw4$^{TT}$ | 14 (33) | 8 (15) | 2.81 | .03[a] |
| 3DL1 + HLA-Bw4$^{I}$ | 13 (31) | 19 (36) | 0.80 | .6 |
| 3DL1 + HLA-Bw4$^{II}$ | 10 (24) | 17 (32) | 0.66 | .4 |
| 3DS1 + HLA-Bw4$^{I}$ | 9 (21) | 12 (23) | 0.93 | .9 |
| 3DS1 + HLA-Bw4$^{II}$ | 6 (14) | 12 (23) | 0.57 | .3 |

The KIR-HLA interaction suggested by literature were analyzed and reported in the table.

Abbreviations: CMV, cytomegalovirus; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor; OR, odds ratio.

[a] Statistical significance ($P < .05$).

[b] Marginal statistical significance ($P < .1$).

**Table 4. Logistic Regression Model to Predict the Occurrence of Symptomatic Infection in AB/BB Haplotype Subjects (n = 95)**

| Variable | Code | β | SE | *P* Value | OR (95% CI) |
|---|---|---|---|---|---|
| HLA-Bw4$^{T}$ | 0: Absent | | | | |
| | 1: Present | 1.02 | 0.47 | .03 | 2.79 (1.13–7.16) |
| HLA-Bw4$^{I}$ | 0: Absent | | | | |
| | 1: Present | −0.91 | 0.63 | n.s. | 0.40 (.10–1.30) |
| (Conditioning variables) | | | | | |
| Sex | 0: female | | | | |
| | 1: male | . . . | . . . | n.s. | . . . |
| Age | 0: <36 y | | | | |
| | 1: ≥36 y | . . . | . . . | n.s. | . . . |

Sex and age did not enter into the final model after the stepwise procedure.

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; n.s., not significant; OR, odds ratio; SE, standard error.

The effect of HLA-Bw4-T is possibly mediated by an interaction with the inhibitory KIR3DL1 and is almost exclusively observed in the Bx haplotype subjects.

# Conclusions

This genetic association study shows that immunocompetent adult subjects possessing the KIR AA haplotype or the HLABw4-T allele are more susceptible to develop a symptomatic acute disease after primary CMV infection.

This is consistent with studies performed in the transplantation setting.

# A constant dream of humankind is to stop, postpone, and/or reverse the ageing process.

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Magic In The

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REVIEW

# Interventions to Slow Aging in Humans: Are We Ready?

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## Summary

**The workshop entitled "Interventions to Slow Aging in Humans: Are We Ready?" was held in Erice, Italy, on October 8–13, 2013, to bring together leading experts in the biology and genetics of aging and obtain a consensus related to the discovery and development of safe interventions to slow aging and increase healthy lifespan in humans. There was consensus that there is sufficient evidence that aging interventions will delay and prevent disease onset for many chronic conditions of adult and old age. Essential pathways have been identified, and behavioral, dietary, and pharmacologic approaches have emerged. Although many gene targets and drugs were discussed and there was not complete consensus about all interventions, the participants selected a subset of the most promising strategies that could be tested in humans for their**

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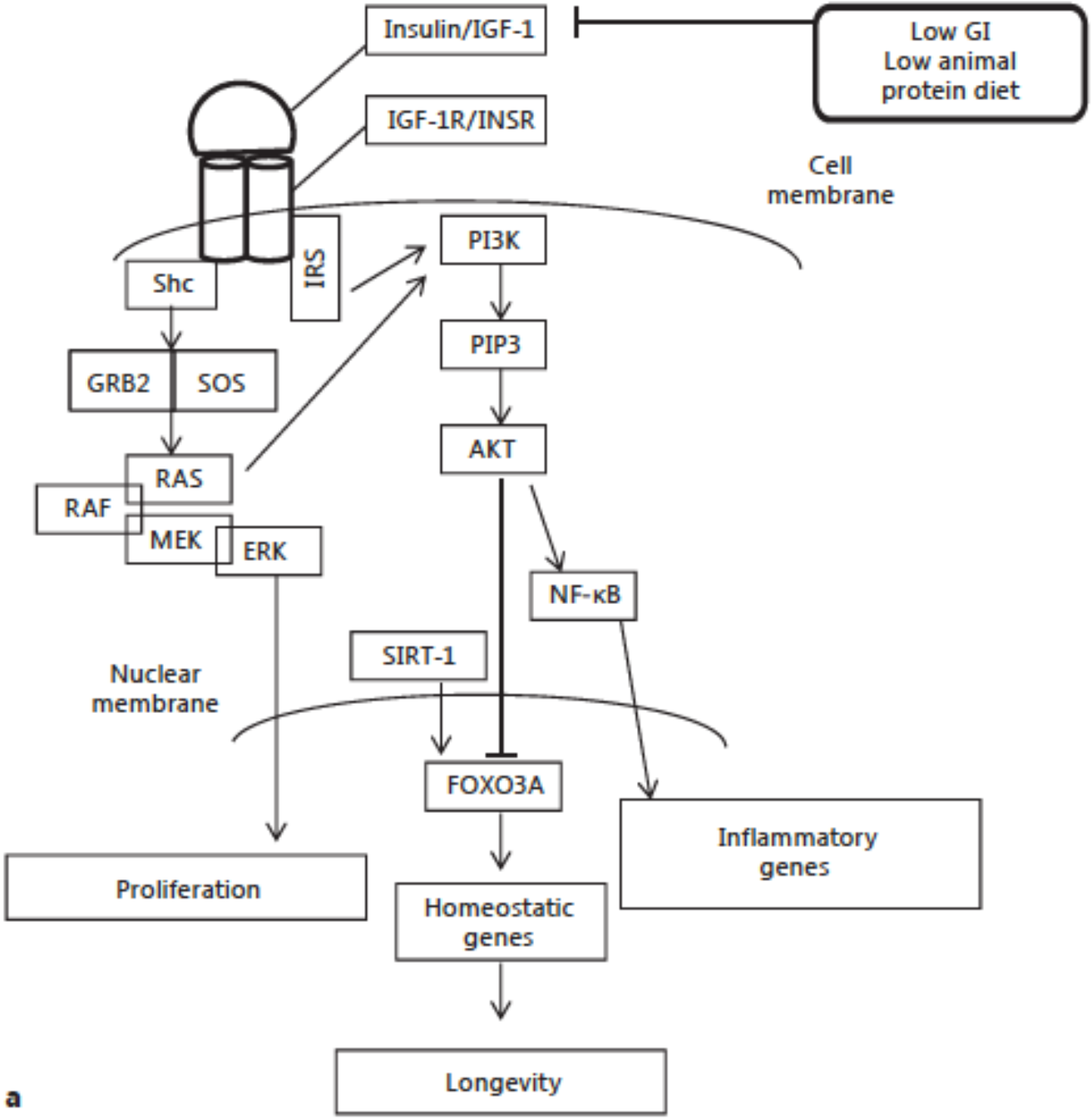
**Fig. 2.** Nutrient-sensing pathways modulate lifespan in humans by interacting with the environment. **a** Insulin/IGF-1 signalling pathway. Insulin (or IGF-1) binds its receptor (IRS) and interacts with PI3K. PI3K activates the second messenger PIP3. PIP3 messenger leads to the activation of AKT that inhibits FOXO3A, preventing the transcription of homeostatic genes. Also, SIRT1 can act on FOXO3A. Moreover, the insulin/IGF-1 signalling determines the activation of the RAS pathway leading to its mitogenic effect. In addition, AKT activates the NF-kB pathway allowing the transcription of inflammatory genes. MedDiet with low GI and/or with low protein intake may reduce the IGF-1 levels and may down-regulate the insulin/IGF-1 pathway leading to the transcription of homeostatic genes and stopping the mitogenic effect of RAS. This favours survival and longevity.

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**Mediterranean Diet and Healthy Ageing: A Sicilian Perspective**

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# Mediterranean Diet and Healthy Ageing: A Sicilian Perspective

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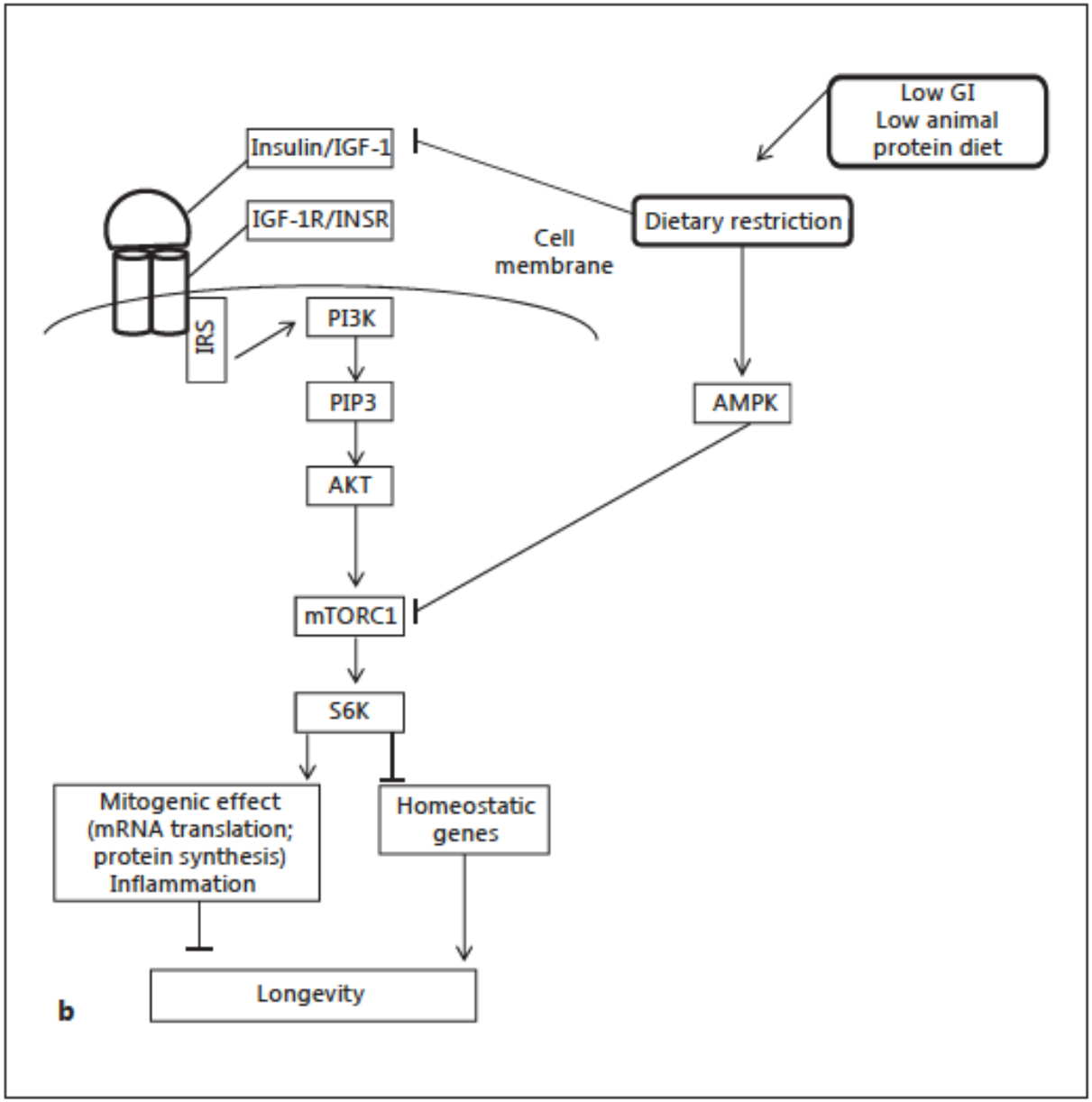


**Fig. 2.** Nutrient-sensing pathways modulate lifespan in humans by interacting with environment. **b** mTOR pathway. AKT determines the inhibition of mTOR complexes. These molecules, via the activation of S6K, determine mRNA translation and protein synthesis with mitogenic and pro-inflammatory effects. Moreover, they inhibit the transcription of homeostatic genes (i.e. genes encoding antioxidant proteins such as catalase or superoxide dismutase or genes promoting autophagy). Dietary restriction, with low GI, low animal protein and a reduction in calorie intake, activates AMPK that inhibits mTORC1 favouring longevity. It means that MedDiet could influence longevity via mTORC1 and insulin/IGF-1 pathway down-regulation (references in the text). IRS = Insulin-responsive substrate; PI3K = phosphatidylinositol 3-kinase; PIP3 = phosphatidylinositol 3-phosphate; SIRT-1 = silent mating type information regulation 1; RAS = rat sarcoma; GI = glycaemic index.

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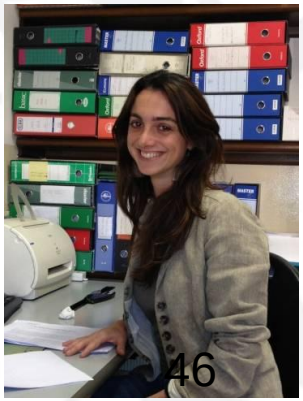



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