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16th IHIW: Immunogenetics of Aging

E. Naumova*, M. Ivanova*, G. Pawelec†, I. Constantinescu‡, K. Bogunia-Kubik§, A. Lange§, F. Oguz¶, K. Ozdilli¶, C. Franceschi, C. Caruso††, M. Mishra‡‡ & D. Middleton§§**

Summary

Ageing is a process characterised by progressive loss of function in multiple different organ systems, such as the nervous, endocrine and immune systems. Current data showing that ageing processes may be associated with alterations in the immune system suggest that some of the genetic determinants of senescence might be polymorphic genes that regulate immune responses. The 'Immunogenetics of Aging' programme was a component introduced in the 14th International HLA and Immunogenetics Workshop (IHIWS) and developed further within the 15th and 16th. The aim of this component was to determine the contribution of immune genes to successful ageing and an increased capacity to reach the extreme limits of lifespan. Within the 16th IHIWS, new populations were included, and the number of samples analysed was increased. Analysis was focused on innate immunity genes (KIR and MBL2) and their correlation with CMV serostatus. Collaborative studies suggested that both activating and inhibitory KIR and functionally relevant MBL2 haplotypes are important factors for control of CMV infection in the elderly and therefore for chronic low-grade inflammation. Results showed that these genes might be predictive biomarkers in ageing and longevity. Prevalence of MBL2 haplotypes determining absence of the protein (LYPB, LYQC and HYPD) was observed in elderly people with a higher CMV antibody titre. The high CMV titre was also associated with a decreased frequency of the activatory KIR2DS5 and A1B10 haplotypes in elderly. Due to the role of KIR and low or deficient MBL haplotypes in viral infections, these genetic markers could be considered as indicators of a need for CMV prophylaxis at younger age and therefore increased probability of longer lifespan.

* Department of Clinical Immunology, University Hospital Alexandrovska, Sofia, Bulgaria, † Center for Medical Research, University of Tubingen, Tubingen, Germany, ‡ Fundeni Clinical Institute Centre for Immunogenetics and Virology, Bucharest, Romania, § Department of Clinical Immunology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland, ¶ Department of Medical Biology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey, ** Department of Experimental Pathology, University of Bologna, Bologna, Italy, †† Dipartimento di Biopatologia e Metodologie Biomediche, Universit`a di Palermo, Palermo, Italy, ‡‡ National Reference Laboratory, New Delhi, India and §§ Transplant Immunology, Royal Liverpool University Hospital, Liverpool, UK

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Correspondence: E. Naumova, Department of Clinical Immunology, University Hospital Alexandrovska, 1.G. Sofiisky Street, 1431 Sofia, Bulgaria. Tel: +359 2 9230 690; Fax: +359 2 9230 496; E-mail: immunology@abv.bg

Introduction

Ageing is a process characterised by progressive loss of function in multiple different organ systems, such as the nervous, endocrine and immune systems. The changes in the immune system are impaired generation of lymphoid progenitors in the bone marrow, thymic involution, loss of capacity for clonal expansion, shrinkage of the T-cell repertoire, prevalence of highly differentiated effector T cells and reduction of naïve T cells, cytokine misbalance, impaired humoral immunity, impaired function of NK cells and other innate immune cells involved in primary immune responses. The clinical consequences of ageing are greater susceptibility to infections, higher severity of the infections leading to higher mortality, partial loss of vaccine efficiency, higher autoreactivity and tumorogenesis (Fulop, 2011). From a socio-economic standpoint, the ageing population is becoming an important issue in the Western world. It is expected that in the next two decades people aged 65 and older will comprise at least 20% of the population (Gaskell *et al.*, 2008). This rapid skewing in the age structure of western populations confers significant stress to healthcare systems as ageing is associated with increased prevalence of medical conditions requiring chronic management and leading to functional dependence. As all these appear to be inevitable consequences of ageing, it is of great importance to understand factors that affect human longevity and ways to improve quality of life.

A strong genetic component of longevity has become apparent from studies with a variety of organisms. Twin studies in humans revealed the magnitude of the genetic influence on lifespan, while centenarian and family studies suggest enrichment of longevity-promoting genes. Such studies showed that somewhere between 15% and 30% of lifespan is controlled by our genes (Herskind *et al.*, 1996; Mitchell *et al.*, 2001). Studies on centenarians, on the other hand, suggest that they owe their good fortune to their inheritance of a small number of powerfully acting longevity genes. Several groups of genes have been investigated for their possible association with human longevity. These include inflammatory and immune-related factors, stress response elements, mediators of glucose and lipid metabolism, components of DNA repair and cellular proliferation and mitochondrial DNA haplogroups. Currently, there is a consensus that many aspects of senescence are characterised by increased inflammatory status, leading to extended tissue damage. Chronic inflammation has been associated with several age-related diseases, including atherosclerosis, obesity, type 2 diabetes and cancer (Franceschi *et al.*, 2000; Cavallone *et al.*, 2003; Chamorro, 2004; Dandona *et al.*, 2004). The extent to which age-associated changes depend on the genetic background needs to be established. Current data showing that ageing processes may be associated with alterations in the immune system suggest that some of the genetic determinants of senescence might be polymorphic genes that regulate immune responses. These genes include those of the most highly polymorphic system known, the human leucocyte antigen (HLA) genes, as well as genes encoding 'atypical' HLA-like molecules (CD1, etc.); killer-cell immunoglobulin-like receptor genes (KIR); leucocyte Fc receptor genes; cytokine and cytokine receptor genes; Toll-like receptor gene family; TNF-receptor associated factors; and mannose-binding lectin 2 (MBL2). Although there are some reports on the possible association of these genes with human longevity and age-related diseases, their impact still remains to be established through collaborative studies on different populations.

The 'Immunogenetics of Aging' programme was a component introduced in the 14th International HLA and Immunogenetics Workshop (IHIWS) and developed further within the 15th and 16th. The aim of this component was to determine the contribution of immune genes to successful ageing and an increased capacity to reach the extreme limits of lifespan. The following sample groups were collected: families with long-lived members (octogenarians and nonagenarians) and unrelated elderly individuals compared with ethnically matched young controls. As part of 14th and 15th IHIWS, the effect of classical HLA class I and class II loci and cytokine polymorphisms in regulatory and/or coding regions, with a possible impact on the level of gene expression of pro- and anti-inflammatory cytokines, was analysed (Naumova *et al.*, 2007, 2011). Some preliminary data on the impact of innate immunity genes such as KIR and MBL2 in the Bulgarian population were generated.

Materials and methods

During the 16th IHIWS, the number of samples collected was increased, and new populations were included. Two sample groups were included in the component 'Immunogenetics of Aging': unrelated healthy elderly individuals and ethnically matched young controls. Elderly individuals were characterised according to the SENIEUR protocol (Ligthart *et al.*, 1984). The unrelated young controls were characterised according to the JUNIEUR protocol (Ligthart *et al.*, 1984). Data on eight populations (Bulgarian, Romanian, Polish, Turkish, Italian, Irish, Danish and Indian) are included with a total number of 973 elderly, 778 young controls and 12 families with long-lived members, including 17 unrelated elderly (65–90 years) and 23 family members (18–57 years) analysed. During the 16th IHIWS, two new populations, Danish and Indians, were included and additional 371 healthy randomly selected elderly individuals (aged 65–99) and 385 young controls were included.

Innate immunity genes KIR and MBL2 and their possible correlation with CMV status were analysed within the 16th IHIWS. KIR genotyping was performed by PCR sequence-specific oligonucleotide (PCR-SSO) and PCR sequence-specific primers (PCR-SSP) methods. For the analysis of MBL2 haplotypes, including six SNPs: rs 1100312 [-619 C/G (L/H)], rs 7096206 [-290 G/C (Y/X)], rs 7095891 [-66 C/T (P/Q)], rs 5030737 [cdn 52 C/T Arg>Cys (A/D)], rs 1800450 [cdn 54 G/A Gly>Asp (A/B)], rs 1800451 [cdn 57 A/G Gly>Glu (A/C)] associated with the protein level, a Luminex-based method was applied. CMV status was assessed by ELISA, and CMV viral load was estimated by RT-PCR.

Allele frequencies were estimated by maximum-likelihood analysis using the Arlequin program v1.1 (Ivanova *et al.*, 2008). Standard deviations were calculated from 100 bootstrap iterations. Hardy–Weinberg equilibrium was tested by a hidden Markov chain with 100 000 steps, implemented in the Arlequin program. Arlequin software was also used to estimate maximum-likelihood three-locus haplotype frequencies from genotypic data through an expectation-maximisation (EM) algorithm (Slatkin & Excoffier, 1996). Haplotypes were confirmed by inheritance in families. Comparisons across different age groups and groups with different CMV status were assessed by the chi-squared test or Fischer exact test when appropriate. A *P*-value lower than 0.05 was considered to indicate a significant difference between groups. Bonferroni correction for multiple comparisons was applied where appropriate.

Results and discussion

HLA

Results from 14th and 15th IHIWS showed positive association of human longevity with HLA-DRB1*11 and HLA-DRB1*16 haplotypes that are protective for autoimmune diseases. During the 16th IHIWS, these data were further extended by analysis of Asian Indian population. Results in this population showed statistically significant negative association of longevity with B*15 (elderly AF-0.097; controls AF-0.307; $P < 0.001$), C*07 (elderly AF-0.250; controls AF-0.548; $P < 0.0001$) and C*15 (elderly AF-0.132; controls AF-0.575; $P < 0.0001$) specificities.

MBL2 haplotypes

It is well established that the serum levels of MBL – the mediator of complement activation – are highly variable and are genetically determined. Three independent single nucleotide polymorphisms (SNPs): cdn 52 (C/T; Arg/Cys, allele D); cdn 54 (G/A; Gly/Asp, allele B); cdn 57 (G/A; Gly/Glu, allele C) disrupt the collagenous structure of the protein and dramatically reduce serum MBL concentrations (Madsen *et al.*, 1994). Any of these mutations (B, C or D) is referred to as O, whereas the wild type is referred to as A. In addition to exon 1 SNPs, three regulatory variants in the promoter and in the 5 = UTR regions at positions: -619 (C/G, allele L/H), -290 (G/C; allele Y/X) and -66 (C/T; allele P/Q) also influence final serum MBL concentration. Combinations of these six SNPs result in widespread haplotypes, determining different serum MBL levels (Madsen *et al.*, 1995, 1998): deficiency (haplotypes LYPB, LYQC, HYPD); low level (haplotype LXPA); intermediate level (haplotype LYPA); and high level (haplotypes HYPA and LYQA). MBL allele and haplotype distribution is quite diverse in different populations, and MBL deficiency is one of the most common immune deficiencies. Studies in different ethnic groups (Ivanova *et al.*, 2008) showed a high, almost similar (25–28%) proportion of haplotypes associated with MBL deficiency. Different hypotheses were proposed to explain the unusually high frequency of MBL2-deficient genetic variants worldwide, including a protective effect of the low serum MBL against infections especially those with intracellular bacteria or parasites (Hoal-Van Helden *et al.*, 1999) or alternatively, reduction of deleterious effects of complement-mediated inflammation. Although many individuals with low MBL levels are asymptomatic, clinical symptoms are apparent in the presence of additional immune defects. Genetically determined MBL deficiency has been associated with an increased susceptibility to many infections in cases of compromised adaptive immunity, for example, in early childhood (Cedzynski *et al.*, 2004; Wiertsema *et al.*, 2006), in cases of inherited and acquired immune deficiencies, after chemotherapy (Mlle *et al.*, 2006) or after HSCT (Granell *et al.*, 2006). Furthermore, MBL insufficiency is thought to influence some autoimmune diseases such as systemic lupus erythematosis (SLE; Takahashi *et al.*, 2005) and rheumatoid arthritis (Graudal & Madsen, 1998). Considering these, we hypothesised that MBL2 gene polymorphism might be a possible factor contributing to human longevity. To test this hypothesis in cross-sectional and longitudinal studies, we have tested six MBL2 SNPs in the following populations Bulgarians, Romanians and Danish. Results showed a tendency towards a decreased frequency of the LYPA haplotype associated with intermediate serum levels and an increased frequency of LYPB determining MBL deficiency in elderly compared with young controls, although the difference did not achieve statistical significance (Table 1). Additionally, the two haplotypes LYPD and HXPA, the functional relevance of which remains to be established, were observed both in elderly and young controls. As ageing is associated with chronic low-grade inflammatory activity possibly related to CMV infection, we further correlated MBL2 polymorphism and CMV antibody level. Data from the 16th IHWS on the increased number of subjects confirmed our previous results (Naumova *et al.*, 2011) showing prevalence of MBL2 haplotypes determining

Table 1. The most frequent mannose-binding lectin 2 (MBL2) haplotypes in elderly compared to young controls – Bulgarian, Romanian, Danish population

Haplotype								HF – Bulgarians		HF – Romanians		HF – Danish	HF – combined	
-619	-290	-66	cdn 52	cdn 54	cdn 57	MBL2 level		Elderly	Controls	Elderly	Controls	Elderly	Elderly	Controls
G	G	C	C	G	G	High	HYPA	0.289	0.276	0.283	0.150	0.024	0.226	0.213
C	G	T	C	G	G	High	LYQA	0.108	0.173	0.233	0.081	0.102	0.151	0.127
C	G	C	C	G	G	Interm	LYPA	0.024	0.000	0.175	0.314	0.025	0.069	0.214
C	C	C	C	G	G	Low	LXPA	0.217	0.265	0.091	0.260	0.359	0.203	0.262
C	G	C	C	A	G	Absence	LYPB	0.205	0.133	0.075	0.051	0.119	0.161	0.092
G	G	C	T	G	G	Absence	HYPD	0.157	0.192	0.083	0.000	0.056	0.116	0.102
C	G	T	C	G	A	Absence	LYQC	0.000	0.029	0.000	0.000	0.000	0.000	0.020
C	G	C	T	G	G	?	LYPD	0.000	0.010	0.017	0.017	0.038	0.021	0.013
G	C	C	C	G	G	?	HXPA	0.060	0.010	0.000	0.067	0.092	0.062	0.047

absence of the protein (LYPB, LYQC, and HYPD) in elderly people with a CMV antibody titre of >20 IU μL^{-1} (haplotype frequency −0.37) compared with those with a titre of <20 IU mL^{-1} (haplotype frequency −0.26).

KIR polymorphism

Within the 15th and 16th IHWS, we also focused on KIR polymorphism as another innate immunity gene system possibly involved in longevity. In addition to the data from the 15th IHWS showing an possibly increased frequency of 2DS3 (elderly GF-40.9%, controls GF-25.0%, $P < 0.05$, Pc = ns) and KIR2DL5 (elderly GF-59.1%, controls GF-45.0%) in the aged compared with the young controls in the Irish population, extended data in Bulgarians showed of a higher frequency of KIR2DL2 and lower KIR2DL3 and 2DS1 in elderly people (Fig. 1). Additionally, analysis of haplotypes determined according to the model of Middleton *et al.* (2005) showed increased frequencies of A1B1, A1B3 and A1B4 and a decreased frequency of A1B10 the elderly group (Table 2).

Several studies reported an association between KIR genes and the outcome of some viral infections (Martin & Carrington, 2005; Kulkarni *et al.*, 2008; Romero *et al.*, 2008; Zaia *et al.*, 2009). Data on the role of KIR in the control of CMV infection are limited and include studies mainly in transplant recipients (Zaia *et al.*, 2009). Studies on the increased number of subjects in the Bulgarian population within the 16th IHIWS confirmed previous data (Naumova *et al.*, 2011) that a higher CMV-specific IgG antibody level in elderly individuals was associated with a decreased frequency of the activatory KIR2DS5 and A1B10 haplotypes. Interestingly, the presence of the A1B10 profile in combination with HLA-DR11 and DR16 (alleles contained within longevity-associated HLA haplotypes) was noted in individuals with low-grade CMV seropositivity (<20 IU mL^{-1}). It is very likely that epistatic interactions between certain HLA and KIR haplotypes may contribute to initiating dynamic immune responses facilitating successful ageing, while some haplotype combinations could be related to suboptimal immune response and to reduced lifespan.

At the end of the 16th IHIWS, a consensus was reached on recommendations for future progress. It was concluded that additional samples and data from different populations should be collected. It is also critical to incorporate additional, possibly functionally relevant polymorphisms in other immune genes: for example, KIR/Ligand combinations, TLR4, CD14,

Table 2. Frequencies of killer-cell immunoglobulin-like receptor haplotypes in Bulgarians

	Haplotype frequencies (%)	
Haplotype	Elderly individuals	Young controls
AxAx	4.5	0
A1B1	**13.6**	**0**
A1B2	0	5.9
A1B3	**13.6**	**5.9**
A1B4	**13.6**	**5.9**
A1B6	4.5	5.9
A1B5	9.1	5.9
A1B10[a]	**9.1**	**17.6**
A3B2	4.5	0
A3B4	4.5	0
A3B10	0	5.9
A4B5	0	5.9
A1Bx	9.1	29.4
AxBx	13.6	0
A3Bx	0	5.9
AxB4	0	5.9

[a]In combination with DRB1*11 and DRB1*16 increased in individuals with CMV <20 IU mL^{-1}.
Values in bold show a trend with non significant *p* value.

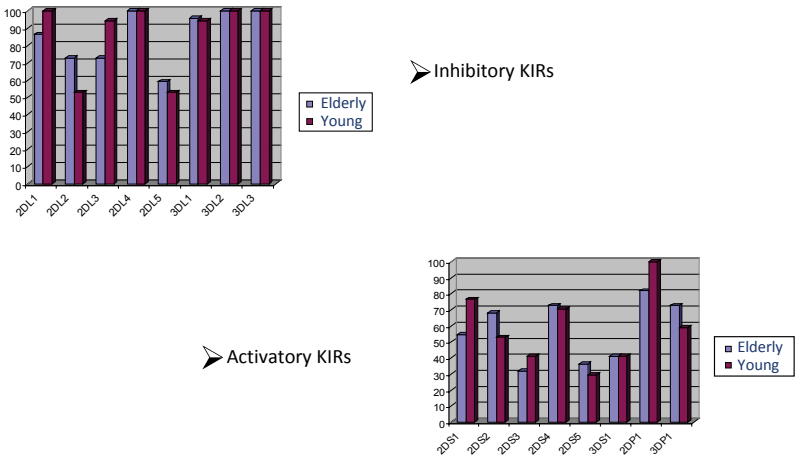

Figure 1. Killer-cell immunoglobulin-like receptor (KIR) gene frequencies in elderly compared to young individuals from the Bulgarian population.

CCR5 and MMP3. Another possible study would be to include genes associated with the constant decrease in hematopoietic function with age: ageing of the stem cell niche rendering less efficient HSC self-renewal, decline in the relative size of the HSC population, less efficient mobilisation properties, skewed differentiation profiles with age and also to include genes related to a frequent cause of bone marrow failure associated with a lack of genome stability which leads to myelodysplastic syndrome and overt leukaemias.

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