'Immunogenetics of Aging': report on the activities of the 15th International HLA and Immunogenetics Working Group and 15th International HLA and Immunogenetics Workshop

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Abstract

'Immunogenetics of Aging' is a component that was first included in the 14th International HLA and Immunogenetics Workshop (IHIWS) and developed further within the 15th Workshop. The aim of this component was to assess the impact of human leukocyte antigen (HLA) genes, cytokine genes, and some innate immunity genes such as killer-cell immunoglobulin-like receptors (KIRs) and mannose-binding lectin 2 (MBL2) in successful aging and their contribution to the better understanding of immune dysfunction in old age. Within the 15th IHIWS new populations were included in the analysis. Additional cytokine gene polymorphisms were assessed and innate immunity genes were analyzed for possible relevance in longevity. The results showed that longevity might be associated with anti-inflammatory cytokine gene profiles, decreased frequency of interleukin-10 (IL-10) and transforming growth factor-B1 haplotypes associated with a low level of gene expression, and increased frequency of haplotypes determining a high level of expression. Extended tumor necrosis factor-A and IL-12B genotypes were also likely relevant to longevity. Data also showed that innate immunity genes are associated with susceptibility to infections in the elderly and showed that these genes might be an important genetic marker in aging. Decreased frequencies of KIR2DS5 and A1B10 haplotypes, and an increased proportion of MBL2-deficient haplotypes were found in the group with higher cytomegalovirus-specific IgG antibody levels. Together, these studies emphasize the relevance of genes regulating immune functions in maintaining human longevity and stress the importance of further clarifying their impact on successful aging.

The aging process is very complex and longevity is a multifactorial trait, which is determined by genetic and environmental factors and the interaction of 'disease' processes with 'intrinsic' aging processes. Twin studies show that genes account for 15–30% of human lifespan determination (1, 2). Many studies showed that there are genetic differences between centenarians and the general population. Additionally, family studies showed that the siblings of centenarians have a higher likelihood of reaching old age than the general

population (3). It is hypothesized that the level of immune response influences longevity, and that this should therefore be associated with genes regulating immune functions (4). The 'Immunogenetics of Aging' program is a component included within the 14th International HLA and Immunogenetics Workshop (IHIWS) developed further within the 15th. The aim of this component was to determine the contribution of immune genes to successful aging and an increased capacity to reach the extreme limits of lifespan. Two main

data sets were collected: families with long-lived members (octogenarians and nonagenarians) and unrelated elderly individuals compared with ethnically matched young controls. As part of 14th IHIWS, the effect of classical human leukocyte antigen (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 analyzed. Results showed that longevity in the populations studied is positively associated with DRB1*11- and DRB1*16-associated haplotypes and with increased anti-inflammatory cytokine genotypes (5). During the 15th IHIWS, the number of samples collected was increased, polymorphisms in cytokine genes and innate immunity genes such as killer-cell immunoglobulin-like receptor (KIR) and MBL2 were included in the analysis. Linkage and association analyses were performed in order to identify extended immunogenetic profiles that could be relevant to a better understanding of the mechanisms contributing to successful aging and longevity.

Several previous studies had showed that cytokine dysregulation is associated with different disorders including age-related disease. Increased interleukin-6 (IL-6) levels in the elderly (6) are associated with the development of frailty and susceptibility to diseases (7). Additionally, several investigators reported increased *ex vivo* capacity of macrophages from the elderly to produce IL-10 and increased transforming growth factor-β (TGF-β) levels (6). On the other hand, decreased capacity to produce interferon-γ (IFN-γ) was observed in cells from the elderly upon stimulation. Studies over the last few years have shown that polymorphisms in the regulatory regions of cytokine genes could affect gene transcription and cause different cytokine production levels (8). Although some investigators have shown associations of cytokine gene polymorphism with longevity, data are still controversial and inconclusive (9–16).

This report will review the analyses of data collected by the Immunogenetics of Aging Working Group. Results on cytokine gene polymorphisms and innate immunity genes in unrelated elderly and controls and families with long-lived members are presented. Finally, the report will summarize the future plans of the Immunogenetics of Aging Working Group.

Two main data sets were included in this study: unrelated healthy elderly individuals and ethnically matched young controls, and families with long-lived members. The following selection criteria were used to identify families for the study: extended families with a family history of at least two generations with long-lived members (octogenarians and nonagenarians) including elderly individuals, their children, and grandchildren; availability of sufficient demographic data; and data on family history of diseases. Elderly individuals (in family-based analyses and unrelated case-control analyses) were characterized according to the SENIEUR protocol (17). The unrelated young controls were characterized according to JUNIEUR protocol (17).

Table 1 Number of elderly and young individuals from each population and testing performed within the component Immunogenetics of Aging

Population	Number of individuals			Genetic markers analyzed
	Elderly	Young	Families	
Bulgarians	60	100	12 families (three generations)	HLA[a], IL-2, IL-6, IL-10, TNF-A, TGF-B1, IFN-γ, IL-12B, KIR, MBL2
Turkish	237	90		HLA[a], TNF-A, TGF-B1, IL-10, IL-6, IFN-γ
Romanians	32	44		HLA[a]
Polish		129		HLA[a], TNF-A, IL-6, IFN-γ, IL-10
Italians	410	20		IL-10, IFN-γ, TNF-A, IL-2, IL-12B, KIR
Irish	100	100		KIR

HLA, human leukocyte antigen; IFN-γ, interferon-γ; IL, interleukin; KIR, killer-cell immunoglobulin-like receptor; MBL2, mannose-binding lectin 2; TGF-B1, transforming growth factor-B1; TNF-A, tumor necrosis factor-A.
[a]HLA analysis was performed within the 14th IHIWS.

Data on six populations (the Bulgarian, Romanian, Polish, Turkish, Italian, and Irish) are included in the component Immunogenetics of Aging. During the 15th IHIWS, an additional 572 healthy randomly selected elderly individuals (aged 65–99) were collected. A total of 602 elderly, 393 young controls, and 12 families with long-lived members, including 17 unrelated elderly (65–90 years) and 23 family members (18–57 years) were analyzed within this component. The number of elderly and young individuals from each population and the testing performed are given in Table 1.

In addition to the genetic markers HLA-A, -B, -DRB1, -DQB1 loci, and single nucleotide polymorphisms (SNPs) in cytokine genes possibly associated with the level of gene expression [tumor necrosis factor-A (TNF-A); TGF-B1; IL-10; IL-6; IFN-γ, IL-2] analyzed as a part of the 14th IHIWS (5), extended genotypes in TNF-A, IL-12B, and TGF-B1 genes were assessed and new genetic markers (MBL2 and KIR) were included within the 15th IHIWS.

The additional single nucleotide gene polymorphisms in cytokine genes TNF-A, TGF-B1, and IL-12B were assessed by polymerase chain reaction-sequencing based typing (PCR-SBT) methods. 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 associated with the protein level, a Luminex-based method was applied (18).

Allele frequencies were estimated by maximum-likelihood analysis using the Arlequin program v1.1 (19). 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-maximization (EM) algorithm (20). Haplotypes were confirmed by inheritance in families. Comparisons across different age groups 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.

In this report we detail the major findings of the collaborative study within the 15th IHIWS. Analysis of cytokine gene polymorphisms in this study showed no statistically significant differences between the elderly individuals and the control groups from different populations for genotype frequencies of the generally pro-inflammatory cytokines IL-2, IL-6, and IFN-γ. However, associations with longevity were observed for IL-10 haplotypes (Table 2), which were determined by EM algorithm which had been validated in 12 families. The IL-10 haplotype (-1082G, -819C, and -592C) associated with high-level cytokine gene expression was significantly more prevalent among healthy elderly to young controls ($P_c <$ 0.05). These results were observed in both the Bulgarian and the Turkish populations. Additionally, in Bulgarians the IL-10 haplotype (-1082A, -819T, and -592A), possibly related to a lower level of gene expression, was found with slightly lower frequency in the elderly. In the Turkish population, a decreased frequency of another low-level IL-10 haplotype (-1082A, -819C, and -592C) was observed in the elderly ($P_c < 0.05$). The observations imply that longevity might be associated with anti-inflammatory cytokine gene profiles.

Analysis of the extended TNF-A genotypes showed significant differences between elderly individuals and young controls. A genotype, possibly related to low-level expression, was more frequent among healthy elderly Bulgarians compared with young controls (Table 3).

Analysis of genotypes of anti-inflammatory TGF-B1 also showed possible association with aging. The genotype TGF-B1 (codon 10) T/T; (codon 25) G/G, related to high levels of gene expression, was increased in elderly compared with young controls ($P < 0.05$). In contrast there was a trend to a lower frequency in elderly of the genotype TGF-B1 (codon 10) C/C; (codon 25) G/G, associated with low protein level (Table 4). Analysis of extended TGF-B1 genotypes in

Table 2 IL-10 haplotypes in elderly and young controls

	Haplotype frequency			
	Bulgarians		Turkish	
IL-10 (-1082; -819; -592) haplotypes	Elderly ($n = 60$)	Young ($n = 60$)	Elderly ($n = 237$)	Young ($n = 90$)
ACC (low)	0.297	0.336	0.261**	0.364**
ATA (low)	0.238*	0.358*	0.291	0.250
GCC (high)	0.466**	0.306**	0.487**	0.386**

IL, interleukin.
*$P < 0.05$, $P_c =$ ns; **$P < 0.01$, $P_c < 0.05$.

Table 3 Extended TNF-A genotypes in elderly Bulgarians compared with ethnically matched controls

TFN-A genotypes					Genotype frequency	
					Elderly ($n = 60$)	Young ($n = 50$)
-1031	-863	-857	-308	$+489$		
C/C	A/A	C/C	G/G	G/G	0.028	0.040
C/C	C/A	C/C	G/G	G/G** (low)	0.083	0.000
C/C	C/C	C/C	G/G	G/G	0.056	0.000
C/C	C/C	C/T	G/G	G/A	0.028	0.040
T/C	A/A	C/T	G/G	G/A	0.028	0.000
T/C	C/A	C/C	G/A	G/G	0.056	0.000
T/C	C/A	C/C	G/G	G/G* (low)	0.167	0.040
T/C	C/A	C/T	G/G	G/A	0.028	0.000
T/C	C/C	C/C	G/G	G/G	0.028	0.080
T/C	C/C	C/C	G/A	G/G	0.000	0.040
T/T	C/C	C/C	A/A	G/G	0.028	0.040
T/T	C/C	C/C	G/A	G/A	0.028	0.000
T/T	C/C	C/C	G/A	G/G	0.111	0.080
T/T	C/C	C/C	G/G	G/G	0.083	0.200
T/T	C/C	C/T	G/A	G/A	0.056	0.080
T/T	C/C	C/T	G/A	G/A	0.111	0.120
T/T	C/C	C/T	G/G	G/G	0.028	0.000
T/T	C/C	T/T	G/G	A/A	0.056	0.120

TNF-A, tumor necrosis factor-A.
*$P < 0.05$, $P_c =$ ns; **$P < 0.01$, $P_c < 0.05$.

Table 4 TGF-B1 genotypes in elderly compared with young controls

TGF-B1 genotypes		Genotype frequency			
		Bulgarians		Turkish	
Codon 10	Codon 25	Elderly ($n = 60$)	Young ($n = 40$)	Elderly ($n = 237$)	Young ($n = 90$)
C/C	C/C	0.000	0.000	0.004	0.011
C/C	G/C	0.069	0.108	0.034	0.000
C/C	G/G	0.172	0.216	0.131*	0.230*
T/C	C/C	0.000	0.000	0.008	0.000
T/C	G/C	0.103	0.081	0.085	0.046
T/C	G/G	0.396	0.459	0.347	0.483
T/T	G/C	0.000	0.000	0.017	0.000
T/T	G/G	0.259	0.135	0.373*	0.23*

TGF-B1, transforming growth factor-B1.
*$P < 0.05$, $P_c =$ ns.

Bulgarians showed that genotype TGF-B1 (codon 10) C/C; (codon 25) G/G; (-988) C/C; (-800) G/G was decreased, while the genotype TGF-B1 (codon 10) T/C; (codon 25) G/G; (-988) C/C; (-800) G/A was increased in elderly compared with the controls (Table 5). Based on codon 10 and 25 these genotypes are associated with low- and high-level gene expression, respectively. As the positions -988 and -800 are located in the promoter region of the gene they could also possibly modulate the expression of TGF-B1.

Analysis of IL-12B extended genotypes did not show any statistically significant associations despite of the slightly increased frequencies of (1188) C/C; (2124) A/A genotype in elderly compared with young controls in both Bulgarians and

Table 5 Extended TGF-B1 genotypes in elderly Bulgarians compared with ethnically matched controls

TGF-B1 genotypes				Genotype frequency	
Codon 10	Codon 25	−988	−800	Elderly ($n = 60$)	Young ($n = 40$)
C/C	G/C	C/C	G/G	0.034	0.056
C/C	G/C	C/T	G/G	0.034	0.000
C/C	G/G	C/C	G/G* (low)	0.085	0.254
C/C	G/G	C/T	G/G	0.034	0.000
T/C	G/C	C/C	G/A	0.017	0.028
T/C	G/C	C/C	G/G	0.068	0.056
T/C	G/G	C/C	G/A* (high)	0.051	0.000
T/C	G/G	C/C	G/G	0.272	0.306
T/C	G/G	C/T	G/G	0.034	0.028
T/T	G/G	C/C	G/A	0.017	0.000
T/T	G/G	C/C	G/G	0.167	0.139
T/T	G/G	C/T	G/G	0.017	0.000

TGF-B1, transforming growth factor-B1.

*$P < 0.05$, P_c = ns.

Table 6 KIR gene frequencies in elderly and young controls from the Irish population

	KIR gene frequency			
	Young controls ($n = 100$)		Elderly individuals ($n = 100$)	
KIR gene	n	(%)	n	(%)
2DL2	49	49.0	52	55.9
2DL3	93	93.0	82	88.2
2DS1	36	36.0	42	45.2
2DS2	49	49.0	52	55.9
2DS3*	25	25.0	38	40.9
2DS4	94	94.0	88	94.6
2DS5	28	28.0	32	34.4
3DS1	39	39.0	42	45.2
3DL1	94	94.0	87	93.5
2DL5	45	45.0	55	59.1
2DL4, 3DL2, 3DL3	100	100	93	93

KIR, killer-cell immunoglobulin-like receptors.

*$P < 0.05$, P_c = ns.

Italians. The functional relevance of the different distribution of this genotype remains to be clarified.

As a part of the 15th IHWS some innate immune genes such as KIR and MBL2 associated with susceptibility to infections have been assessed as possible biomarkers related to aging. KIR gene polymorphism was studied in two populations – one in the north (Irish) and another in the south (Bulgarians). Comparison of KIR genotype distribution in the Irish cohort shows significantly increased frequencies of KIR2DS3 ($P < 0.05$, P_c = ns) and KIR2DL5 in the aged subset compared with the young controls (Table 6), suggesting that these KIR genes may play a role in successful aging in this population. On the other hand, only a trend toward a higher frequency of KIR2DL2 and lower KIR2DL3 and 2DS1 was observed in aged Bulgarians. Further, analysis of KIR haplotype distribution was performed in Bulgarians according to the model of Middleton et al. (21). Increased frequencies of A1B1 [haplotype frequency (HF) elderly = 0.136; HF controls = 0], A1B3 (HF elderly = 0.136; HF controls = 0.059), and A1B4 (HF elderly = 0.136; HF controls = 0.059) and a decreased frequency of A1B10 (HF elderly = 0.091; HF controls = 0.176) profiles were found in the elderly group but the differences were not statistically significant. Studies are now emerging supporting the effect of the presence or absence of particular KIR genes on the outcome of certain viral infections (22–28). However, little is known about the role of KIR genetic background in the control of cytomegalovirus (CMV) infection, which is strongly implicated as part of the 'immune risk profile' in longitudinal studies of elderly Swedes (29). It has been shown that a donor KIR profile, containing either KIR2DS2 and 2DS4 or having ≥5 activating KIR genes is predictive for a low risk of CMV reactivation in the recipient after hematopoietic stem cell transplantation (24). In a case study of a child with a novel immunodeficiency syndrome KIR2DL1 expression on all natural-killer (NK) cells was associated with recurrent CMV infection (25). As aging is associated with chronic, low-grade inflammatory activity thought to be amplified by persistent infection with CMV, the correlation between KIR and CMV status in the elderly was analyzed (Figure 1). Decreased frequencies of KIR2DS5 ($P > 0.05$) and A1B10 haplotypes ($P < 0.05$, P_c = ns) were found in the group with higher (>20 IU/ml) CMV-specific IgG antibody levels. Taking into consideration that KIR2DS5 is an activating receptor and the A1B10 haplotype is characterized by more inhibitory and less activating KIRs in comparison with the other AB profiles (except A1B1), different mechanisms leading to the same effects on persistent CMV could be applicable as previously suggested in other viral infections (26–28). 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). Along these lines, an association between DRB1*1101/DQB1*0301 and HCV clearance has also been reported (22). A possible explanation for this finding could be that epistatic interactions between certain HLA and KIR haplotypes may contribute to the generation of a more effective immune response and suppression of a latent viral infection. As KIRs act in combination with their ligands further efforts to define associations between KIR/HLA ligand combinations and CMV status will greatly enhance our ability to elucidate the role of KIRs in successful aging.

It is well established that MBL serum levels are highly variable and that they are genetically determined by six SNPs. Three independent SNPs: cdn 52 (C/T; Arg/Cys, allele D); cdn 54 (G/A; Gly>Asp, allele B); and cdn 57 (G/A; Gly>Glu, allele C) disrupt the collagenous structure of the protein and dramatically reduce serum MBL concentrations (30–32). Any of these mutations (B, C, or D) is referred to as O, while the wild type is referred to as A. In addition to exon one SNPs,

Inhibitory KIRs
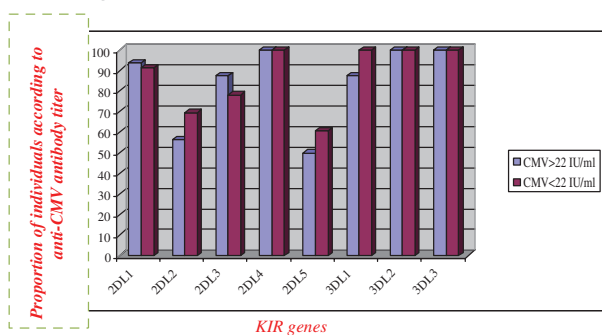
Activatory KIRs
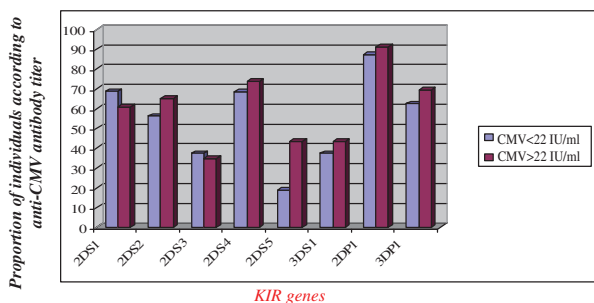
Figure 1 Correlation of killer-cell immunoglobulin-like receptor (KIR) gene distribution and the anti-cytomegalovirus (CMV) antibody titer. Analysis of the distribution of KIR genes showed decreased frequencies of KIR2DS5 ($P = $ ns) in the group with higher ($>$20 IU/ml) CMV-specific IgG antibody levels.

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 (33, 34): deficiency (haplotypes LYPB, LYQC, and 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, the clinical consequences of which have been extensively studied over the past few years. As a part of the component 'Immunogenetics of Aging' we also sought associations of MBL2 polymorphisms and CMV infections in elderly. Our preliminary results showed that in the elderly, MBL2 haplotypes determining absence of the protein (LYPB, LYQC, and HYPD) were more frequent in those with a CMV antibody titer of $>$20 IU/μl compared with those with a titer of $<$20 IU/ml (37% *vs* 26%, p-ns) (Figure 2). Therefore, this might be studied further as a useful additional relevant marker in aging.

In summary, our previous results suggest that longevity is associated with HLA haplotypes shown to be protective for diseases. Additionally, genotypes related to an enhanced anti-inflammatory profile could be positively associated with longevity. Extended genotypes are more informative in aging because of their possible functional significance. Inheritance of extended haplotypes in families with long-lived members allowed us to identify immunogenetic profiles that could be predictive of longevity. Innate immunity genes are associated with susceptibility to infections and might be relevant genetic markers in aging and longevity.

At the end of the 15th IHIW, a consensus was reached on recommendations for future progress. A major challenge in evaluating the large number of genes that are likely to be picked up by the new techniques now available will be to distinguish which of the many differences are involved in the aging process, and which are a consequence of it. It was concluded that additional samples/data from different ethnic groups should be collected and additional, possibly functionally relevant polymorphisms in other genes such as TLR4, CD14, CCR5, and MMP3 should be included. Expression studies of IL and KIR genes would contribute to a more precise assessment of the influence of the genetic background on human longevity.

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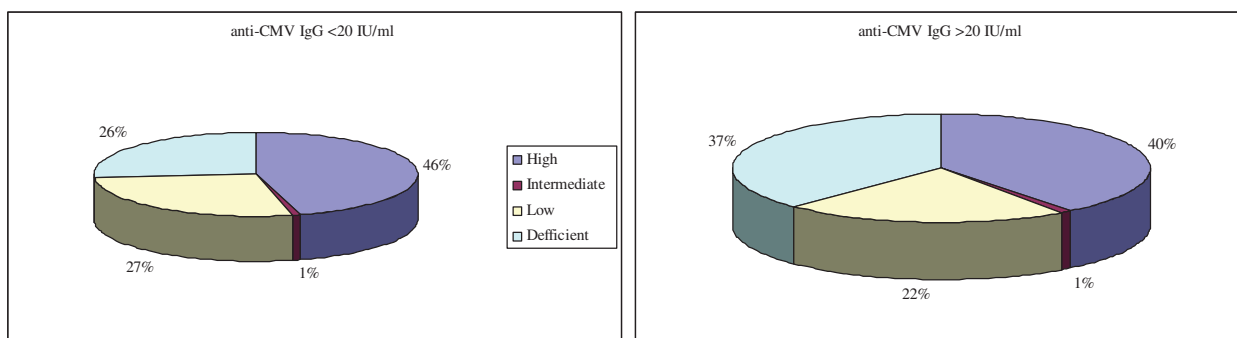
Figure 2 Mannose-binding lectin 2 (MBL2) haplotype distribution and anti-cytomegalovirus (CMV) antibody levels in the elderly: high level of MBL – HYPA, LYQA; intermediate level of MBL2 – LYPA; low level of MBL2 – LXPA; and deficiency of MBL2 – LYPB, LYQC, HYPD.

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