LETTER TO JMG

Inflammation, genetics, and longevity: further studies on the protective effects in men of *IL-10* –1082 promoter SNP and its interaction with *TNF-α* –308 promoter SNP

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Ageing is associated with chronic, low grade inflammatory activity leading to long term tissue damage, and systemic chronic inflammation has been found to be related to mortality risk from all causes in older persons.[1] Also, the genetic constitution of the organism interacting with systemic inflammation may cause defined organ specific illnesses. Thus, age related diseases such as Alzheimer's disease (AD), Parkinson's disease, atherosclerosis, type 2 diabetes, sarcopenia, and osteoporosis, are initiated or worsened by systemic inflammation, suggesting the critical importance of unregulated systemic inflammation in the shortening of survival in humans.[1–3] Accordingly, proinflammatory cytokines are believed to play a pathogenetic role in age related diseases, and genetic variations located within their promoter regions have been shown to influence the susceptibility to age related diseases, by increasing gene transcription and therefore cytokine production.[3 4]

Conversely, genetic variations determining increased production of anti-inflammatory cytokines or decreased production of proinflammatory cytokines have been shown to be associated with successful ageing, suggesting a role for the control of the inflammatory state in the attainment of longevity. As recently reported, the distribution of +874T→A interferon(IFN)-γ,[5] −174C→G IL-6,[6] and −1082G→A interleukin(IL)-10[7] single nucleotide polymorphisms (SNPs) has been shown to be different in centenarians than in younger people.[5–7] The +874T allele, involved in high production of the proinflammatory cytokine IFN-γ, was found less frequently in centenarian women than in control women.[5] Also, the proportion of subjects homozygous for the G allele at the –174 *IL-6* locus, characterised by high serum levels of the proinflammatory cytokine IL-6, was significantly decreased in centenarian men.[6] Conversely, the presence of the –1082 GG genotype, suggested to be associated with high production of the anti-inflammatory cytokine IL-10, was significantly increased in centenarian men in comparison with younger male subjects.[7] These gender related effects are difficult to explain. However, it is well known that men and women may follow different strategies to reach longevity and that the number of centenarian women always outnumber centenarian men, the ratio between women and men ranging from 4/1 to 7/1, with the exception of Sardinia.[8 9]

It is rather simplistic to examine the influence of a single cytokine in isolation. In fact, cytokines interact in networks in which the functions of one cytokine are modified, modulated, or substituted by another one(s).[4] In particular, IL-10 and tumour necrosis factor (TNF-α), a classical proinflammatory cytokine, have complex and opposing roles,[10 11] and an autoregulatory loop appears to exist in which TNF-α stimulates IL-10 production which, in turn, reduces TNF-α synthesis.[12 13] Furthermore, their synergistic role in the control of immune-inflammatory responses has been hypothesised.[14] To gain further insight into the relation between inflammatory cytokines and longevity, we investigated the frequency of the −1082G→A *IL-10* promoter SNP in a larger cohort of 72 centenarian men, including 25 typed in a previous study,[7] and the interaction of this polymorphism with −308A→G *TNF*-α SNP.

Key points

- Many aspects of ageing involve inflammatory processes. We evaluated the association with longevity of alleles of *IL-10* and *TNF-α*, known to have opposite functions in inflammatory reactions, IL-10 acting predominantly as an anti-inflammatory and TNF-α as a proinflammatory factor.
- The number of male centenarians homozygous for the –1082G genotype, suggested to be associated with high IL-10 production, was significantly increased in comparison with younger control subjects. No significant differences were observed between women and controls. The genotypic frequencies of the *TNF-α* promoter SNPs 308G and 308A, suggested to be associated with low and high TNF-α production respectively, were not significantly different between centenarians and controls. The evaluation of combined *IL-10* and *TNF-α* genotypes showed that there was a significant increase of the "anti-inflammatory" (*IL-10* –1082GG/*TNF-α* –308GG) genotype in centenarian men over controls.
- Inflammatory markers predict disability and mortality in elderly cohorts and a persistent inflammatory state has been proposed to be involved in the causal pathway of certain age related chronic conditions. Thus, it is intriguing that the possession of an "anti-inflammatory" genotype is significantly increased in male centenarians.

MATERIALS AND METHODS

DNA samples were obtained from 72 unrelated centenarian men (25 of whom were part of a previous study)[7] and 102 centenarian women from central and southern Italy. DNA samples from healthy unrelated controls (115 men and 112 women, aged 22-60 years), matched for geographical origin, were also collected. Written informed consent for enrolment in the study and for personal data management was obtained from all the subjects according to Italian laws.

Blood specimens were collected in tripotassium EDTA sterile tubes and immediately stored at −70°C. Genomic DNA extraction was carried out and DNA stored at −20°C for the *IL-10* and *TNF*-α gene analysis. Complete linkage of allele –819C with allele –592C and of allele –819T with allele –592A,

Table 1 Genotypic frequencies for 1082G→A *IL-10* promoter gene SNP and for 308G→A *TNF-α* promoter gene SNP in 115 controls and in 72 centenarian men

	Controls (%)	Centenarians (%)
–1082 *IL-10*		
AA	19 (16)	5 (7)
AG	64 (56)	34 (47)
GG	32 (28)	33 (46)
–308 *TNF-α*		
AA	1 (1)	1 (1)
AG	31 (27)	15 (21)
GG	83 (72)	56 (78)

All groups were in Hardy Weinberg equilibrium (p>0.05). A significantly different distribution of *IL-10* genotypes was observed between controls and centenarians (p=0.019 by χ^2 test). Similarly, the frequency of SNP –1082G was significantly increased in centenarians over controls (0.69 *v* 0.56, p = 0.01 by χ^2 test). It is noteworthy that this significant difference was observed both in the previous sample of 25 centenarians (0.76 *v* 0.56, p = 0.012 by χ^2 test) and in the new sample of 47 centenarians (0.66 *v* 0.56, p = 0.035 by χ^2 test). No significant different distribution of *TNF-α* –308 genotypes was observed between controls and centenarians by χ^2 test and the frequency of SNPs –308G→A was not significantly different between centenarians and controls by χ^2 test.

and the presence of only three different allele combinations, –1082G, –819C, and –592C GCC, ACC, and ATA, are characteristic of the *IL-10* polymorphisms in white populations.[15] In the previous study, only –1082 SNP was shown to be associated with longevity, so in the present study we limited ourselves to the analysis of the –1082 G→A SNP. This biallelic polymorphism (GeneBank accession number: 790448) was identified using ARMS-PCR as previously described.[7] The biallelic polymorphisms at –308 (G→A) (GeneBank Accession number CR941560) of the *TNF*-α gene was identified also using the ARMS-PCR method as previously described.[16] Allele typing was confirmed according to Wilson *et al*.[17]

TNF-α and *IL-10* genotypic and allelic frequencies were evaluated by gene count. The data were tested for the goodness of fit between the observed and expected genotype values (χ^2 test) and their fit to Hardy-Weinberg equilibrium. χ^2 tests (3×2 tables or 2×2 tables) were performed to calculate significantly different genotype or allele distributions between centenarians and controls.

RESULTS

Table 1 shows the frequency of homozygous and heterozygous genotypes for –1082 G→A *IL-10* SNP and for –308 G→A *TNF*-α in our sample of Italian centenarian men and sex matched controls. The number of centenarians homozygous for the –1082G genotype was significantly increased in this cohort of centenarian men in comparison with younger control subjects (p=0.019). Accordingly, the SNP distribution between centenarians and controls was significantly different, with the –1082G allele increased in centenarians (0.69 *v* 0.56, p=0.01 by χ^2 test). As already reported,[7] no significant differences were observed in women, between centenarians and controls (–1082G, 0.62 *v* 0.59, p=NS by χ^2 test). The genotypic frequencies of the *TNF*-α promoter SNP of centenarian and control men were not significantly different. Accordingly, the frequency of SNP –308G→A was not significantly different between centenarians and controls (χ^2 test). The same was observed in the group of centenarian women (–308G, 0.88 *v* 0.79, p=NS by χ^2 test).

As far as cytokine production is concerned, the *IL-10* and *TNF*-α polymorphisms considered are functionally important. Thus, centenarians and controls were classified into four groups, as shown in table 2. We compared the frequency of the genotype "low *TNF*-α and high *IL-10*" producer, "anti-inflammatory genotype"[14] with the other genotypes in centenarian and control men. There was a significant increase of this "anti-inflammatory genotype" in centenarians over controls (36% *v* 21%; p=0.038 by χ^2 test). In women, there were no significant differences in the distribution of the "functional" genotypes between centenarians and younger subjects (32% *v* 21, p=NS by χ^2 test).

DISCUSSION

The main result of this study was that the *IL-10* gene SNP –1082G→A had significant influence on the attainment of longevity in men, both separately and in association with the *TNF*-α gene SNP –3082A→G. It is noteworthy that the *IL-10* frequency was analysed in a large cohort of male centenarians, thus validating our preliminary results on a smaller sample of centenarian men.[7] This result is remarkable, taking into account that centenarian men are relatively rare, being many fewer than centenarian women.[8]

IL-10 and TNF-α are cytokines, which have complex and predominantly opposing roles in the inflammatory responses.[10 11] In fact, the principal routine function of IL-10 is to limit and ultimately terminate inflammatory responses,[10] whereas TNF-α determines strength, effectiveness, and duration of local and systemic inflammatory reactions.[11] Stimulation of human blood samples with bacterial lipopolysaccharide showed large interperson variations of IL-10 and TNF-α production, suggesting a genetic component of approximately 75% and 60%, respectively.[18] Interperson differences in the regulation of IL-10 and TNF-α production may be critical with respect to the final outcome of an inflammatory response, that is within physiological limits or pathological ones.[18] Several polymorphisms located close to or within the *IL-10* and *TNF*-α genes have been shown to be associated with transcription levels. The best documented of these polymorphisms are the *IL-10* gene promoter polymorphisms –1082 G→A[7 16 19–23] and a G→A transition at position –308 in the promoter region of *TNF*-α.[11 13 24 25] In the presence of allele –1082A, stimulation of lymphocytes with concanavalin A resulted in lower IL-10 production than in allele –1082A negative cells.[14 21] Also, the effects of this allelic promoter region difference (relative to the transcription start site) on *IL-10* gene activation have been confirmed in reporter gene assays.[20] Concerning *TNF*-α, there are also data relating the rare –308A allele to a higher transcriptional activity than the common –308G allele,[11 13 24 25] although a lack of such an association has also been reported in non-white populations, depending on the HLA ancestral haplotype carrying this SNP.[12 24] However, the functional relevance of these SNPs has been shown by their involvement in determining susceptibility to immune-inflammatory diseases (for *IL-10* –1082G→A,[14 20 26–35] for *TNF*-α[11–13 24 25 36–42]).

Table 2 Functional *IL-10*/*TNF-α* genotypes

	TNF-α –308A carrier (A/A, A/G)	*TNF-α* –308G/G
IL-10 –1082A carrier (A/A, A/G)	Low IL-10, high TNF-α	Low IL-10, low TNF-α
IL-10 –1082G/G	High IL-10, high TNF-α	High IL-10, low TNF-α

IL-10 –1082A carriers are classified as "low IL-10 producers"; *IL-10* –1082GG homozygotes are classified as "high IL-10 producers"; *TNF-α* –308A carriers are classified as "high TNF producers"; *TNF-α* –308GG homozygotes are classified as "low TNF producers". For references, see text.

The isolated assessment of cytokine genotypes may be misleading without considering other interacting cytokines. In vivo control of TNF-α synthesis is complex and downregulated by anti-inflammatory cytokines including IL-10.[12 13] The potential biological importance of this finding was highlighted by a recent study showing a strong association between the combined low *IL-10*/high *TNF*-α genotype and early graft rejection in heart transplant recipients.[14] Other recent studies have shown the complementary importance of IL-10 and TNF-α in patients with bacterial sepsis and have indicated an increased mortality in patients with a high plasma IL-10 to TNF-α ratio, that is, in patients with an anti-inflammatory phenotype (unfortunately in this study patients were not genotyped).[43] Thus, in the light of different pathophysiological situations, it is difficult to assume a beneficial or detrimental effect of *IL-10/TNF*-α genotypes separately or in combination. So, inflammatory genotypes may be both friends and enemies. In fact, they are an important and necessary part of the normal host responses to pathogens, but the overproduction of inflammatory cytokines might cause immune-inflammatory diseases and eventually death. In particular, an anti-inflammatory genotype might be highly advantageous in the last decades of life owing to the chronic proinflammatory status, which develops in all the subjects with age. This phenomenon that we called inflamm-ageing is more evident in men than in women.[14] This could be a possible explanation for the higher frequency of the anti-inflammatory genotype we found in very old male subjects. We can also predict that the presence of the "high *IL-10*/low *TNF*-α" genotype could be favourable in protecting against age related diseases such as AD and in elderly stroke.[35 45 46]

Finally, we would like to stress that our study has a number of possible limitations. Since this study was performed with Italian centenarians, we do not know whether the results can be extended to populations of other ethnic origins. Notably, in a Finnish population, *IL-10* and *TNF*-α promoter allele and haplotype frequencies were not different between nonagenarians and controls.[47] Moreover, no significant association between polymorphisms of some cytokines and longevity has been found in the Sardinian population.[48] On the whole, these findings suggest that cytokine/longevity associations might have a population specific component, being affected by the population specific gene pool as well as by gene-environment interaction. Another limitation of the study was the lack of data regarding plasma levels of IL-10 and TNF-α, but the functional relevance of the polymorphisms studied is well known (see above). Besides, it may be argued that a Bonferroni type adjustment should be performed to correct for the testing of multiple polymorphisms. If this correction is carried out, none of the polymorphisms would have been significantly associated. However, this correction is too stringent and has the potential to ignore important observations[49] and, in any case, for studies on centenarians the number of cases is very hard to increase.

However, inflammatory markers predict disability and mortality in elderly cohorts and a persistent inflammatory state has been proposed to be involved in the causal pathway of certain age related chronic conditions.[1 3 50] Thus, it is intriguing that the possession of an "anti-inflammatory" genotype is significantly increased in male centenarians.

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