TCR V β REPERTOIRE IN AN ITALIAN LONGEVAL POPULATION INCLUDING CENTENARIANS.

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ABSTRACT

During the last years, the hypothesis that aging and diseases are two distinct phenomena, and that successful aging is possible for most humans, has been put forward.

We studied the TCR V β repertoire of T lymphocytes of healthy longevals and centenarians as crossing point of genetic predisposition and environmental effects to longevity, using the Spectra-typing method.

TCR V β 1, V β 8, and V β 20 were found to be expanded in the longeval population, compared with the younger control population.

This repertoire can have been shaped by the selective action of particular HLA alleles, or by the clonal expansion of specific T cell clones, able to modulate the immune response to endogenous and exogenous antigens. Moreover, the skewed V β usage and the clonal expansion seem to be the effects of physiological changes occurring with aging and not pathological signs of malignity.

INTRODUCTION

Healthy centenarians are attracting much interest as they provide the best example of successful aging. They are people who have escaped major age-related diseases and reached the extreme limit of human life in good clinical condition. In most cases, histories of centenarians reveal them to be free of cancer, dementia, diabetes, cardiovascular diseases, and cataracts. Furthermore, in order to reach such an advanced age, they should be equipped with well-preserved and efficient immune- and defense mechanism, and optimal

combination of an appropriate lifestyle and genetic background (1,2).

The TCR (T Cell Receptor) is a protein structure on the surface of T lymphocytes, able to recognize the peptide/MHC complexes on the membrane of the Antigen Presenting Cells (APCs), and is composed of covalently linked variable α and β chains, non-covalently associated with the CD3 complex (3). The genes encoding the β chain undergo somatic recombination in a manner similar to immunoglobulin molecule (4), resulting in the recombination of V(D)J and C segments. The nibbling of germline nucleotides and/or addition of nucleotides at the V-D-J junction sites further increases this diversity (5). The complementarily determining region 3 (CDR3), encompassing the V-D-J junction, displays the most extensive nucleotide diversity and is thought to encode a region involved in the contact of the TCR with the antigenic peptide (6).

In this report, we present the analysis of the TCR V β segments expressed by T lymphocytes of extremely longeval individuals and centenarians, compared with a younger control population, as crossroad of genetic and environmental influence on predisposition to longevity.

MATERIALS AND METHODS

The association between TCR V β repertoire and predisposition to longevity has been studied comparing a population of 25 healthy longevals (age ranges between 95 and 109 years, mean 101 years), including 19 centenarians, with 24 healthy individuals (mean age 35 years) as controls. The longeval individuals were selected according to the criteria for gerontological studies proposed by the SENIEUR protocol (7).

HLA class I antigens were assessed by standard microcytotoxicity test (8).

HLA class II polymorphism was studied by molecular typing. Genomic DNA was isolated from PBMCs by salting out. DNA was used as template in amplification of class II DRB1, DQA1, and DQB1 alleles using sequence-specific primers (PCR-SSP) (Dynal-Unipath), as recommended (9,10).

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Total RNA was extracted from T lymphocytes of longeval and control individuals using Qiagen columns (Qiagen). RNA was reverse transcribed into cDNA in a reaction using MMLV reverse transcriptase primed with oligo (dT)₁₈ (Clontech), as recommended by the manufacturer.

Aliquots of the cDNA synthesis reaction were amplified using 24 primers specific for each V β family and a single C β -specific as previously described (11). As an internal control for the cDNA amount used per reaction, a tube containing sense and antisense primers for the first exon of C β gene was included. The V β -C β PCR products were then subjected to elongation with a fluorophore-labeled C β specific primer (11).

The size and the fluorescence intensity of labeled run off products were determined on a 377 DNA sequencer (Perkin Elmer Applied Biosystem) and analyzed using the ABI PRISM 377 GENE SCAN analysis program (Perkin Elmer Applied Biosystem) (5).

Aliquots of the V β -C β PCR products, corresponding to the V β 1, V β 8, and V β 20 families of longeval and control populations, were subjected to elongation with 13 fluorophore-labeled J β specific primers (11). The size and the fluorescence intensity of the labeled run off products were determined on a 377 DNA sequencer as previously described.

The relative intensity (RI) of each V β family and V β -J β gene segment was calculated (5).

In order to compare the TCR V β family and V β -J β gene segment distributions within the two populations, mean and standard deviation (SD) of the relative intensities were calculated and tested for statistical significance by 2 tails Student' t test, confidence interval 95%.

The association between TCR V β families and HLA alleles was calculated by Fisher' exact test.

RESULTS

TCR V β Spectratyping

The TCR V β repertoires of T lymphocytes from longevals and controls are not superimposable. In both populations some V β families are present with high frequency, 6-10% in terms of relative intensity: V β 2, V β 3, V β 4, V β 5, V β 6, and V β 19.

TCR V β 1, V β 8, and V β 20, however, are prevalent in the longeval population, showing relative intensities ranging from 2 to 3%, whereas relative intensities of the same V β families in the controls hardly reach 1% ($p=0.0122$, $p=0.0074$, and $p=0.0404$, respectively), as Figure 1 shows.

In order to explore the possibility of the presence of clonal expansion within the V β families, the profile and the peak distribution of V β -C β segments within the spectratypes has been analyzed. In absence of immunodepression or chronic antigenic stimulation, the spectratype of a single family shows 6-8 peaks spaced by 3 nucleotides corresponding to in-frame transcripts of the TCR β chain CDR3 region. The area under each peak is proportional to the amount of transcripts of the

corresponding CDR3 size in the sample. Each peak contains multiple distinct sequences. An increase in the height and area of a peak that modifies the bell-shaped CDR3 size distribution provides an estimation of an oligoclonal or monoclonal expansion (5).

Comparing the spectratypes of the TCR V β families in the two populations, a major difference arises. The peak distribution within the spectratypes of control T lymphocytes shows a prevalently gaussian-like profile, as expected, whereas TCR V β families in the T cells from the longeval population display a substantial mono- or bimodal shape, as illustrated in Figure 2.

V β -J β segments spectratyping

Modifications of the bell-shaped pattern of the peak distribution mirror oligoclonal expansion of particular subfamilies and can be better analyzed by V β -J β segments spectratyping. The relative intensity of each peak in the V β -J β spectratype is the measure of the frequency of each V β -J β recombination segment.

J β segment analysis was performed for the TCR V β 1, V β 8, and V β 20 families of both populations. As shown in Figure 3A, within the V β 1 family, the J β 1.4 segment is present with a statistically significant higher frequency in longevals than in controls (0.054 vs. 0.033, $p=0.0212$). Within the TCR V β 8 family, the prevalent segments are J β 1.4 (longevals 0.046, controls 0.028, $p=0.0488$), J β 1.5 (longevals 0.083, controls 0.043, $p=0.012$), and J β 2.7 (longevals 0.131, controls 0.044, $p=0.0014$), as in Figure 3B. Figure 3C shows that the most represented J β segment in the V β 20 family are J β 1.3 (longevals 0.015, controls 0.004, $p=0.0102$), J β 2.2 (longevals 0.128, controls 0.044, $p=0.0118$), J β 2.6 (longevals 0.015, controls 0.002, $p=0.0026$), and J β 2.7 (longevals 0.194, controls 0.136, $p=0.0314$).

Taking all these data together, no preferential usage of any of the J β 1 or J β 2 variants can be pointed out, even though a slightly prevalence of J β 1.4 and J β 2.7 in the longeval population is highlighted.

HLA alleles and TCR V β families association

The possibility that the different TCR V β repertoires in the longevals and controls could be due to the association with different distribution of HLA alleles within the two populations has been explored. No statistically significant association between TCR V β families and HLA class II or class II alleles was evident (data not shown).

DISCUSSION

In the past decades, major theoretical and technological advances have been achieved in the field of immunosenescence. These new approaches have allowed the analysis of the immune system in a much more sophisticated manner than was possible some years ago.

In this paper we explore the TCR V β repertoire of healthy extremely longeval individuals by spectratyping, a method that permits us to detect the finest differences

V beta families

* $P=0.0122$

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* $P=0.0074$

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* $P=0.0404$

Fig. 1: Distribution of the relative intensities of TCR V β families in longevals and controls. The error bars indicate the Standard Deviation value from the mean value of the Relative Intensity displayed by each V β family.

between TCR usage of cells derived from centenarians and those from younger controls.

Our results show that V β 1, V β 8, and V β 20 are represented with higher frequency, in terms of relative intensity, in the longeval population than in controls. This can be considered as the final physiological result of a reshaping process that occurs to the immune system during the whole life, in response to environmental stimuli, on genetic basis.

HLA genes and molecules, effecting the TCR repertoire during the thymic selection, provide the genetic background. No significant association between HLA and TCR V β usage has been displayed in this study. The idea, however, that the distribution of the V β families is determined by the prevalence of some HLA alleles in the longeval population (12,13), cannot be ruled out, since this result might be biased by the low

number of individuals studied compared to the high polymorphism of HLA loci. In fact, has been already described that HLA haplotypes can generally affect T lymphocyte subsets and their function (14).

Upon aging, the clonal composition of peripheral T cell pool may undergo major changes. Antigen recognition causes activation and clonal expansion of naïve T cells, followed by massive death of effectors and differentiation into memory T cells, which go again through rounds of reactivation, clonal expansion and contraction following each subsequent antigen encounter (15).

It has been demonstrated that CD8⁺ T cell repertoire shrinks with age, and expanded CD8⁺ T cell clones become very frequent and already detectable in young adults, being predominant in 70-75 years old donors in both CD45RA⁺ (naïve cells) and CD45RO⁺ (memory and reactivated cells) (15). On the contrary, CD4⁺ T

Fig. 2: Example of different fluorescent profile of selected Vβ families in a longeval (A) and in a control individual (B). The length of the CDR3 region, in base pairs, is represented on the x-axis. The y-axis indicates the fluorescence intensity in arbitrary units.

cells remain polyclonal much longer, and CD4⁺ expanded clones are rare and accumulate almost exclusively in the CD45RO⁺ cell compartment of centenarians (15). Thus, clonal expansion, mostly related to antigen-experienced CD8⁺ T cells, are a relevant characteristic of immunosenescence (16,17).

Peripheral blood lymphocytes of old human beings, like those of old mice, contain large clones of CD8⁺ T cells, carrying prevalently the CD8⁺CD28⁻ phenotype (18). Has been suggested that lack of CD28 expression reflects a compensatory adaptation of the immune system to chronic stimulation during aging (19).

Fig. 3: TCR V β -J β segment distribution within the V β 1 (A), V β 8 (B), and V β 20 (C) families in the longevity and control population. The error bars indicate the Standard Deviation value from the mean value of the Relative Intensity displayed by each V β -J β segment.

Considering these premises, the skewed repertoire seen in centenarians might be attributed to the prevalence of some T lymphocyte subsets in the blood stream. Meaning and role of these clones in elderly are currently an intriguing puzzle.

It has been showed that the vast majority of CD8+CD28- cells in elderly have cytotoxic activity (20), and suggested that repeated exposure to MHC class I-restricted antigens results in clonal accumulation of endstage mature CTLs (21). These lymphocytes, analyzed by flow cytometry, appeared to be resting cells (not expressing CD25, CD38, CD69, CD71, and HLA-DR), bearing markers of cytotoxic activity (CD11b and C57), and having a phenotype compatible with putative memory cells (upregulated CD2 and CD11a, and CD62L absent) (20). Recently, however, a new subset of T cells with regulatory activity has been described within the CD8+CD28- subpopulation (22). They are able to proliferate *in vitro* under strict culture conditions, recognize specifically MHC class I antigens expressed by the APCs used for priming, and are able to suppress T helper cell reactivity to MHC class II/antigen complex on the same APCs. The suppressive effect results from downregulation of CD40, CD80, and CD86 costimulatory molecules on the surface of antigen presenting cells exposed to the suppressors (22). These cells show a restricted, oligoclonal TCR V β repertoire (23).

At this point, can be possible that the presence of these regulatory cells might be responsible for controlling autoimmune phenomena (24), such as low levels of circulating autoantibodies, especially anti-thyroid, seen in centenarians (25).

The idea that aging is characterized by an expanding mosaic of cells with very different replicative histories has been already put forward (16). In fact, telomere shortening, considered the cell internal clock, does not occur in all immune cells and not at the same time (26). A consequence of this molecular and cellular mosaicism could be the presence of a continuous flow of cellular communications enabling the organism to reshape itself in response to the attrition caused by chronic antigen load (16).

Interestingly, one of the TCR V β families prevailing in the old population is V β 8, which has been described to be involved in the pathogenesis of various autoimmune diseases (27-29). What seems to be a contradiction, to find potentially highly autoreactive V β 8 bearing expanded clones in healthy centenarians, can be actually considered sign of the presence of regulatory cells able to control the response of cells primed to the same antigen. Seen in the context of the "Compensatory Adaptation" theory (30), this paradox makes perfect sense: the high, potentially harmful, reactivity showed by V β 8 in the first period of life becomes a defense tool for the aged organism. In fact, according to the evolutionistic theories on aging, immunosenescence is the ultimate proof that the beneficial effect of the immune system, devoted to the neutralization of dangerous agents early in life and adulthood, turns to be

detrimental late in life, a period largely not foreseen by evolution (16,30).

The spectratypes of TCR V β families from old T cells display a non-gaussian like peak distribution, indicating clonal expansion of particular subfamilies. In some extent, this phenomenon can be considered the T cell counterpart of Benign Monoclonal Gammopathy, with no diagnostic suggestion of T cell lymphoma or leukemia (21). Similar clonal expansion has been found in aged mice CD8+CD28- T cells and these clones have no growth advantage over non-expanded cells in culture (18), underlying the non-pathological meaning of the expansion *per se*. This data can be included in a wider group of results indicating a quantitative reduction of the immune system as a whole in aging. The reduction of the "immunological space" is largely consequent to the lifelong antigenic stress and can be considered a common trait of aging (16).

In conclusion, the TCR V β repertoire showed by T cells from healthy aged people and centenarians is the witness of the challenges endured by the organism during more than nine decades of life, and his ability to remodel himself to reach the extreme limit of human lifespan.

ABBREVIATIONS

TCR: T Cell Receptor;
HLA: Human Leukocyte Antigen;
MHC: Major Histocompatibility Complex;
APC: Antigen Presenting Cell;
PCR: Polymerase Chain Reaction;
CTLs: Cytotoxic T Lymphocytes;
PBLs: Peripheral Blood Leukocytes;
PBMcs: Peripheral Blood Mononuclear Cells.

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