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Vaccine 18 (2000) 1591–1595

Vaccine

www.elsevier.com/locate/vaccine

Genetic factors in immunity and aging

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Abstract

Maximum life span is controlled by genes that regulate molecular mechanisms accounting for the synchrony of structural and functional changes in different cells and tissues of each member of a given species. The role of immune response genes was investigated in aging mice genetically selected for high (H) or low (L) antibody response (Biozzi mice). Results from genetic selection of over 1000 mice showed that genes expressed in the immune system affect life span and diseases. In most cases, the life span is longer in H than in L mice whereas the lymphoma incidence is remarkably higher in L than in H mice. Since DNA repair capacity is a property positively correlated with the maximum life span in several mammalian species, DNA repair was studied by use of hydroxyurea, a cell-synchronizing agent, and found to take place in irradiated human PBMC from young and, to a lesser extent, from adult subjects. Conversely, no repair was detected in irradiated PBMC from elderly subjects. DNA damage recognition and repair pathways involve several nuclear proteins, as double strand breaks are firstly recognized by proteins displaying helicase activity, such as ku 70/80, and then repair is carried out under the control of other proteins. Radiation-induced expression of activated ku70/80 proteins, in terms of DNA-binding, was found in PBMC from young-adults but not from elderly subjects. Maintenance of DNA integrity is fundamental for normal immune functions, as suggested by the lack of V(D)J recombination in lymphocytes of knock-out mice deficient in ku 70 or ku 80 protein. However, whether the link between genetic factors and life span is mediated by the performance of the immune system remains to be demonstrated.

Keywords: Aging; Genes; Immunity

1. Introduction

Maximum life span is under genetic control, as suggested by the following observations. (a) All animal species have characteristic rates of aging. (b) The progeny from the cross of two inbred mouse strains has a longer life span than either parental strain (heterosis). (c) In humans, females live longer than males as in most animal species. (d) Parents who die at ages beyond the average life expectancy have children who likewise are more likely to live longer [1].

The genetic control of the aging rate makes unlikely stochastic processes due to random events and favours programmed events encoded in part of the genome, as in a *master clock*. This genetic complex regulates molecular mechanisms accounting for the synchrony of structural and functional changes in different cells and tissues of each member of a given species. Identification of the genes and processes that set the aging rate differently in different species has been attempted by several approaches [2,3].

Different regions of chromosomes 1, 2, 4, 7, 12 and 17 have been found to interact between themselves and with environmental factors to influence the median life span in recombinant mice of 20 inbred strains [4].

2. MHC genes

A limited gene theory of aging has considered the role of the MHC, which controls immunoregulatory

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0264-410X/00/$ - see front matter
PII: S0264-410X(99)00491-0

cell functions and interactions, in age-related alterations, as most of the diseases observed in senescence have an immunological pathogenesis associated with the decline of immune responsiveness to exogenous antigens and increased tendency to autoimmune reactivity. Experiments with congenic mice have shown that, although genetically identical except for the short MHC region, the mice within each set display considerable variation in maximum life span. However, evidence was provided for a complex interaction between a particular allele and the overall genetic background, as a single allele promotes either longer or shorter life span depending upon the several backgrounds [5].

3. Ir genes (Biozzi mice)

The role of genes for immune responsiveness in the aging process has been investigated in mice genetically selected for high (H) or low (L) antibody response (Biozzi mice). Starting from a heterogeneous outbred population, mice have been selected by two-way assortative breeding for maximal or minimal agglutinin response to the selection antigen in each consecutive generation, so that assortative mating of the highest responder mice generated the H line while that of the lowest responder mice the L line.

Five selections have been carried out for agglutinin responses to different antigens. High or low antibody responsiveness resulted from the interaction of alleles, located at several independent loci, which accumulate progressively in H and L mice during the consecutive generations of selective breeding until homozygosity is reached at the selection limit when the interline difference is maximal. All selections displayed multispecific effects as the difference in antibody response was not restricted to the selecting antigen but was common to several unrelated antigens [6].

Mapping analysis in Selection I showed that some segregating loci are linked to genes on chromosomes 2, 4, 8, 10, and 18, but also to genes certainly involved in major immune functions, such as genes on chromosome 6 coding for the TCR, IgK, and CD8, genes on chromosome 12 coding for the IgH, and genes on chromosome 17 coding for the MHC, TNF-α, TNF-β, C2 and C4 [7].

Improvement of the effect of selection was obtained by assortative breeding from two foundation populations, FoH and FoL, each of which was produced by balanced frequency of the gene pools from the H and L lines of Selections I, II, III, IV, and V. After 16 generations of selection for primary or secondary responses to all antigens used in the original five selections, the difference between H and L lines in antibody responsiveness was remarkably amplified and the multispecific effect of selection was generalized to several antigens. These results, obtained in Selection for general primary (GP) and Selection for general secondary (GS) responses, suggest that more genes with upward effects had accumulated in H mice or, rather, more genes with downward effects had accumulated in L mice during both selections [8].

Whether selection breeding for antibody responsiveness also affects life span and tumour incidence was investigated in Selections I, II, III, and GS (Table 1). The life span was longer in H than in L mice whereas the lymphoma incidence was remarkably higher in L than in H mice. These differences between H and L mice were found in Selections I, II, and GS but not in Selection III [9]. The positive correlation between antibody responsiveness and life span was further analyzed in interline hybrids of Selection II and found statistically significant in most of these mouse populations. Moreover, the life span of the last surviving 20%, which is scarcely affected by early disease-induced mortality and mainly influenced by genes acting on the rate of physiologic aging, appeared as a polygenic character regulated by 3–7 independent loci. Of note, long life span was incompletely dominant in the total population but life span was longer and completely dominant in the last surviving 20% [10].

The results from these studies on genetic selection of over 1000 mice show that genes expressed in the immune system play a significant role in conditioning life span and diseases, in most cases. However, it is

Table 1
Antibody response, life span, and lymphoma incidence in H and L responder mice

Selection	Line	No. of mice	Agglutinin titer ($\log_2$)	Mean life span (days $\pm$ SD)	Lymphomas (%)
I	H	23	12.7	723 ± 216	4
	L	47	4.9	562 ± 130	30
II	H	131	11.4	712 ± 148	14
	L	119	5.2	446 ± 110	35
III	H	189	12.7	611 ± 153	12
	L	130	6.2	622 ± 166	12
GS	H	195	12.8	615 ± 134	12
	L	187	3.6	381 ± 161	61

unclear how genetic selection, e.g. against antibody responsiveness, brings about an increased incidence of malignant lymphomas that are the predominant cause of death. The two characters may be controlled by one set of genes with pleiotropic action or by two sets of linked genes. It would be interesting to examine whether the lymphoma incidence could be decreased and the life span prolonged by improving immune responsiveness in L mice. The results from such an experiment should indicate whether the negative effects of the selected genes are mediated by low immune responsiveness or are independent of immune dysfunctions. On the other hand, the hypothesis that age-related immune dysfunctions have a significant impact on life span and diseases is also supported by the study of centenarians, showing that healthy individuals who have reached the extreme limit of human life in good clinical conditions are equipped with well preserved and efficient immune defence mechanisms [11]. However, in this case the immunologic performance and life span may also be independent traits both determined by favorable genetic factors.

4. DNA integrity

Organisms can survive only if their DNA is replicated faithfully and is rescued from chemical and physical damage that would change its coding properties. Since living cells require the correct function of thousands of proteins, each of which could be damaged by a mutation at many different sites of its gene, DNA sequences must be passed on unchanged if progeny are to have a good chance of survival. DNA is a complex organic molecule of finite chemical stability, as not only it suffers spontaneous damage like loss of bases but it also is assaulted by natural chemicals and radiations that break its backbone and chemically alter the bases. Since mutations result when damage changes the coding properties of bases an organism could not survive the natural rate of damage to its DNA without specific enzymatic mechanisms to repair damaged sites. DNA repair is, indeed, so important that a bacterium may devote a considerable percent of its genome to specifying and controlling the enzymes involved.

Over the past two decades, several studies have addressed the relationships between DNA damage, repair and aging, and have suggested an age-dependent accumulation of DNA damage. Using cells from a variety of mammalian species, a positive correlation between life span and the capacity of repairing DNA damage has been demonstrated [12]. In particular, peripheral blood mononuclear cells (PBMC) [13] and epidermal cells [14] from elderly humans have shown decreased DNA repair as compared to adult controls.

4.1. DNA repair

DNA repair was studied by use of hydroxyurea (HU), an agent that synchronizes cells in S phase. We found that DNA repair takes place in irradiated PBMC from young and, to a lesser extent, from adult subjects. Conversely, no recovery was found in PBMC from elderly subjects [15] (Table 2).

The role of DNA repair in cellular aging has recently raised great interest following the identification of cellular proteins that play a key role in DNA damage recognition and repair pathways. The damage is firstly recognized by nuclear proteins displaying helicase activity, and DNA repair is then carried out under the control of other proteins [16].

Eukaryotic cells contain many copies of the helicase ku 70/80 protein dimer. This protein has been described to be involved in the control of DNA replication and transcription and in V(D)J recombination [17,18]. ku 80-deficient mice exhibit severe combined immunodeficiency due to T and B lymphocyte arrest at early progenitor stages [18]. ku 70/80 protein is able to bind not only to DNA ends, but also to other alterations in double-stranded DNA, such as hairpins, nicks and gaps [19]. ku 70/80 protein has also been described to bind to other enzymes of the repair cascade, such as DNA ligase III [20]. Furthermore, ku 70/80 protein is the DNA-binding component of a DNA-dependent protein kinase (DNA-PK) whose catalytic

Table 2
PHA responsiveness and DNA repair[a] after in vitro irradiation of synchronized PBMC from subjects of different ages

Population examined	In vitro treatment of PBMC			Repair Ratio ± SE (95%CL)
	PHA	PHA + HU	PHA + HU + RX	
Young (20–30 years)	10538 ± 438[b]	1009 ± 38	4903 ± 240	4.88 ± 0.22 (4.27–5.49)
Adult (40–50 years)	9301 ± 286	1080 ± 27	2872 ± 99	2.62 ± 0.15 (2.20–3.04)
Elderly (61–89 years)	1704 ± 112	273 ± 18	304 ± 11	1.19 ± 0.06 (0.50–1.83)

[a] Repair is expressed by the ratio: cpm in culture (PHA + HU + RX)/cpm in culture (PHA + HU).
[b] Mean cpm ± SE from 8 subjects.

subunit (DNA-PKcs) can phosphorylate a wide range of nuclear factors [21]. The ability of ku 70/80 protein to recognize and bind to double strand DNA ends suggests that its binding represents an early step in the repair process. The substrates of phosphorylation have only been identified in part and could include components of the transcription and replication machinery and proteins directly involved in the repair process itself. Moreover, the large DNA-PK complex could hold DNA ends in a configuration promoting their rejoining [22]. DNA-PKcs might also regulate the assembly of multiprotein complexes or, alternatively, it might act more indirectly by regulating the expression of genes that are involved in the repair process or in a linked process, such as cell cycle regulation. The latter function of the DNA-PKcs would provide an effective mechanism able to block DNA replication before cells have repaired their damaged DNA [19].

4.2. DNA-binding of the ku 70/80 protein

PBMC from persons of different ages were incubated with PHA for different times (1, 3, 5, 7 or 18 h) of culture. At the end of each incubation time, nuclear extracts were prepared and tested in Electrophoretic Mobility Shift Assay (EMSA). Under our experimental conditions, DNA-binding of the ku 70/80 protein is cumulative, as also evidenced by previous experiments, since the number and density of the bands are related to the protein concentration in the nuclear extract. DNA-binding of the ku 70/80 protein in nuclear extracts of *unirradiated* PBMC from subjects of different ages demonstrates that, after 1 h of mitogen stimulation, the DNA-binding ku is expressed in all nuclear extracts suggesting that aging does not affect the expression of this protein. DNA-binding of the ku 70/80 protein is evident after all times of mitogen activation. In these experiments, a polyclonal rabbit antibody specific for the ku 80 protein was able to supershift all bands.

After 1 h stimulation by PHA, DNA-binding of the ku 70/80 protein is increased by irradiation in PBMC from young and adult, but not from elderly subjects. The densitometric analysis (Table 3) of the upper bands, reported as the ratio between densities after and before PBMC irradiation, indicates that the radiation-induced DNA-binding of the ku 70/80 protein declines with aging.

Table 3
Maximum increase of DNA-binding activity to DNA of ku 70/80 protein after X-ray irradiation of PBMC

Population examined	Ratio between irradiated and unirradiated
Young (20–30 years)	5.34 ± 0.60[a]
Adult (40–50 years)	2.09 ± 0.16
Elderly (61–89 years)	1.05 ± 0.2

[a] Mean ratio $\pm$ SE from 8 subjects.

Our data [15] provide the first evidence for radiation-induced activation of the ku 70/80 protein, in terms of DNA-binding, in PBMC from young-adults but not from old human subjects. Experiments were performed with a moderate dose of X-rays, 500 cGy, to generate a modest concentration of DNA breaks with high probability of an accurate repair. With higher radiation doses, the DNA fragmentation increases but with greater probability that unrelated ends are re-joined, leading to mismatch repair.

The ku 70/80 protein may be involved in both the recognition of breaks generated by X-rays and the activation of cellular events leading to complete DNA repair. The latter mechanism is supported by the evidence that radiation-induced breaks are not simply ligatable and the sequential activation of repair enzymes is required. It is conceivable that after radiation-induced DNA damage, many copies of ku translocate from the cytoplasm into the nucleus, where they bind to double strand breaks. The evidence that the cytoplasmic ku is unable to bind DNA, as indicated by the results of EMSA (not shown), suggests that ku must be activated to bind DNA and this process may result from a phosphorylation/dephosphorylation event or from an association/dissociation with the other components of the DNA-PK complex. Moreover, as radiation-induced breaks block DNA polymerization and repair [23], we cannot exclude a possible role of ku in the activation of enzymes involved in their removal [24].

Mitogen-activated PBMC showed increased DNA repair capacity as compared to unstimulated PBMC (not shown), suggesting the existence of a relationship between proliferative activity and repair capacity. It is not known, however, if the age-related impairment in the proliferative activity leads to reduced DNA repair potential or if the reduction of the DNA repair capacity with aging affects the proliferation of PBMC from elderly subjects.

5. Conclusion

Maintenance of DNA integrity is fundamental for normal immune functions, as suggested by the lack of V(D)J recombination in lymphocytes of knock-out mice deficient in ku 70 or ku 80 protein [18]. The reduced DNA repair capacity of human lymphocytes with aging could play a key role in the deterioration of immune reactivity and contribute to the development of age-associated immune dysfunctions which, in turn, may affect the life span. An interesting approach would be to investigate DNA repair and ku protein activity in H and L Biozzi mice displaying different

immune responsiveness and life span. However, whether the link between genetic factors and life span is mediated by the performance of the immune system remains to be demonstrated.

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