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Genetic control of immune responsiveness, aging and tumor incidence

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Abstract

Age-related alterations of the immune system affect both antibody and cell-mediated immune responses, T-cell responses being more severely affected than B-cell responses. Within the T-cell population, aging leads to replacement of virgin by memory cells and to accumulation of cells with signal transduction defects. Changes in T-cell subsets and in cytokine production profiles may produce suitable conditions for T-cell-mediated disregulation of antibody responses characterized by the production of low affinity and self-reactive antibodies. Also B-cells exhibit intrinsic defects and natural killer (NK) cell activity a profound loss in old mice. Whether age-related immune disfunctions influence life span and tumor incidence has been examined in mice genetically selected for high or low antibody responsiveness. It has been found that genetic selection of vigorous antibody responses in most cases produces mice with longer life span and lower lymphoma incidence. Moreover, the results of genetic segregation experiments indicate that antibody responsiveness and life span are polygenic traits regulated by a small number of the same or closely linked loci. Mice genetically selected for high or low mitotic responsiveness to PHA exhibit low or high tumor incidence, respectively, but no difference in life span, suggesting that T-cell activity is restricted to immune surveillance of neoplastic transformation. Studies on mice genetically selected for resistance or sensitivity to chemical carcinogenesis have uncovered loci that control both resistance to tumor induction and longevity while have no effects on immunity and disease incidence. Thus, the relative role of the immune system in conditioning the duration and the biological quality of life remains to be determined. © 1997 Elsevier Science Ireland Ltd.

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1. The aging immune system

Aging is associated with a progressive decline of immune responsiveness to exogenous antigens and with increasing incidence of autoimmune phenomena [1]. An immunological view of aging suggests that alterations in T- and B-cell populations, leading to immune disfunctions, may affect disease incidence and life span [2].

Age influences both antibody and cell-mediated immune responses, the effects resulting mostly from changes in cell numbers and functions rather than in the cellular milieu [3]. T-cell responses are more severely affected than B-cell responses. T-cell proliferative responses to antigens and mitogens and T-cell-mediated immune responses, such as cutaneous delayed-type hypersensitivity (DTH), mixed lymphocyte reactivity (MLR), cell-mediated cytotoxicity (CMC), decrease with aging [4]. Limiting dilution assays have indicated an age-associated decline in the frequency of responding T-cells, the strength of the response being well preserved in each cell that can respond [5]. Aging negatively affects the membrane structures involved in the early events of T-cell activation [6,7]. T-cells from aged mice exhibit defects in calcium mobilization [8] and protein phosphorilation [9] that lead to changes in signal transduction pathways. The decreased T-cell immunity is also associated with shifts in T-cell subsets and in cytokine secretion profiles. Changes in the splenic T-cell population have been shown to be a consequence of thymus involution [10] and to consist of a gradual decline in cell number with an increase of the $CD4^+$ $CD8^+$ cell ratio, due to a relative decrease in the $CD8^+$ cell number [11]. Although the percent of splenic $CD4^+$ T-cells does not change greatly with age, the composition of this cell subpopulation is quite different in young and old mice. The peripheral pool of $CD4^+$ naive T-cells ($CD44^{low}$ $CD45RB^{high}$), which has been shown to be predominant in young mice, decreases with age while that of memory T-cells ($CD44^{high}$ $CD45RB^{low}$) increases [12,13]. Studies on the role of the thymus in age-related changes of the naive and memory T-cell pools [14] have indicated that the young thymus has greater propensity to provide naive T-cells as compared to the old thymus that, instead, favors the differentiation and maintenance of memory T-cells rather than naive T-cells. Furthermore, young naive T-cells produce mainly IL-2 whereas young memory T-cells mainly IL-4. At variance, in old mice memory T-cells produce twice as much IL-2 as naive T-cells, while the overall level of IL-2 is significantly lower than that in young mice. Moreover, in old mice naive T-cells produce twice as much IL-4 as memory T-cells, while the overall level of IL-4 is at least the same as that in young mice. These findings [14] are consistent with previous observations showing enhancement of IL-3, IL-4, IL-5, IL-10 and IFN-γ production, at the level of cytokine secretion [12,13] and gene expression [15], by $CD4^+$ T-cells from old mice.

The age-related alterations of the peripheral $CD4^+$ T helper cell subsets in terms of cell surface phenotype and cytokine production, as they emerge from the aging thymus, may produce suitable conditions for T-cell-mediated disregulation of antibody responses. Hence, an unbalance within the $CD4^+$ T-cell subpopulation may reduce B-cell proliferation and mutation rate during the antibody response, leading to the production of low affinity antibodies with increased cross-reactivity to self antigens [16–18]. Although in some cases autoantibodies are of the IgM isotype, the respone to many, if not all, autoantigens requires T-cell help [19]. The appearance of autoreactive T-cells in the periphery [20,21] is often concomitant with the infiltration of organs by $CD4^+$ T-cells [22] although evidence of the organ-specific reactivity of these cells is lacking. It is likely that autoimmunity is induced or prevented by the interplay of autoreactive and regulatory $CD4^+$ T-cell subsets [23].

The antibody response to exogenous antigens is decreased in aging, but it is uncertain to what extent this decline reflects intrinsic changes in B-cell functions [24] or changes in the performance of T-cells needed to promote B-cell activation and differentiation [25]. Studies of B-cell hybridomas [26] and single B-cells [27] have indicated that individual cells from aging mice are increasingly likely to produce antibodies with low affinity and cross-reactive specificity for foreign and self antigens. Limited data [28] suggest that aging may lead to major changes in the molecular processes by which antibody genes are assembled and then selected. Impairment of B-cell generation in bone marrow of old mice, as shown at the level of pro- and pre-B-cells [29], may induce qualitative as well as quantitative alterations affecting tolerance and responsiveness of the mature B-cell population.

The ability of accessory cells to support T- and B-cell activation seems unaffected by aging. However, the observed defects in the ability of follicular dendritic cells to process and present immune complexes may contribute to the decline of germinal center formation in old mice [16,30]. Furthermore, the defects in the transport of antigen into lymph node germinal centers by migrating dendritic cells may also contribute to decrease humoral and cell-mediated immunity [31].

Studies of natural killer (NK) cell function in old mice have shown a profound loss of NK cell function when spleen and lymph node cells were assayed, suggesting that a loss of NK cell activity contributes to increasing sensitivity to neoplastic and viral diseases [32,33].

2. The impact of immunity on life span and disease

The relationship between immune disfunctions and the risk of dying from infections or neoplastic diseases has been examined to a great extent with the aim of identifying immunologic indicators with diagnostic and prognostic values. The results of this approach have not been very satisfactory although some combinations of immunologic indices appear to have predictive values [34].

A striking feature of aging is the increase of autoimmune responses, as evidenced by the rise in frequency and concentration of serum autoantibodies [19]. Although this phenomenon has been recognized for many years, its biological significance is still debated. Autoantibodies in the elderly either aid in the removal of senescent cells from the organism [35] or induce cellular damage and thereby contribute to the pathology associated with aging [2]. It should be pointed out, however, that many of the classical autoimmune syndromes exhibit a peak incidence in midlife, and the contribution of autoreactivity to the degenerative and neoplastic diseases of aging is still no more than speculation [1].

Human studies have provided some results which support the possibility that immune disfunctions may contribute to the vulnerability of the elderly to infections and neoplastic diseases and, therefore, increase the risk of mortality. Healthy men were found to have lower peripheral blood lymphocyte counts associated with diminished 1-year survival [36]. However, it was not specified whether the decrease in lymphocyte count represented a loss in one particular T-cell or B-cell subset or was correlated with functional defects. More recently [34], human studies have shown that people aged between 86 and 92 years at the time of the assay were more likely to die in a 2-year follow-up period if they exhibited low T-cell mitotic response, low $CD4^+$ and high $CD8^+$ cell numbers, and low B-cell number as compared to people displaying opposite values in the immunologic tests. Also skin tests for DTH carried out in people 60 years old or over have revealed that anergy is correlated with an increased risk of dying from pneumonia and cancer.

The hypothesis that immunosenescence may contribute to mortality risk is supported by the finding that the proportion of $CD8^+$ T-cells in the peripheral blood of mice at any age is predictive of the remaining life expectancy, the higher the proportion the lower the expectancy [37]. Noteworthily, also high numbers of memory T-cells at 6 months of age were associated with a high risk of early mortality [34].

Immune reconstitution experiments previously reported [1] have provided some results which support the notion that T-cell deficiency in the aged may predipose to infection, as the vulnerability of old mice to polio virus, tuberculosis and Listeria was decreased by administration of T-cells from younger donors. The cellular mechanisms accounting for the age-related increase in susceptibility to viral diseases find some insights in the analysis of human responses to influenza vaccination [38]. It was observed that elderly subjects who exhibited both low scrum IL-2 levels and good production of IL-2 in an in vitro test were as likely to respond well to vaccination as were young controls, whereas poor vaccination responses were typical of elderly subjects with high serum IL-2 levels and low in vitro IL-2 production.

Whether age-related immune disfunctions predispose to tumor incidence and development is still an open question. It seems very likely that the rate of tumor development is tightly coupled to the aging process, but whether the altered immunity in old age is among the coupling mechanisms remains to be established [1]. The demonstration that alleles at immunoregulatory loci influence life span and tumor incidence in segregating populations may provide crucial information for the

analysis of the links connecting immune function to ncoplastic and other late-life diseases [34].

3. The genetic approach

3.1. The Biozzi mice as animal model

Biozzi mice [39], genetically selected for high (H) or low (L) antibody responsiveness, were used to investigate whether genetic selection for H or L immune response brings about changes in life-span and disease incidence.

Starting from distinct foundation populations (F0) of outbred mice, five selections were carried out by two-way assortative breeding for maximal or minimal agglutinin response to natural immunogens (erythrocytes, *Salmonella*, or proteins) 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 (Table 1). H or L 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 at all relevant loci is reached at the selection limit when the interline difference is maximal. The immunogenetic parameters in the five selections exhibited a remarkable similarity (Table 1). Only in selection V was the interline difference greater and attained after fewer generations. The number of independent loci controlling antibody responsiveness differed in the five selections owing to the different nature of the selection antigens and the immunization procedures used [40]. More recently, mapping studies associating polymorphic microsatellite markers with antibody response in selection I have shown that some segregating loci are linked to genes on chromosomes 4 and 8 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 allotypes, and genes on chromosome 17 coding for the MHC, TNFα, TNFβ, C2 and C4 [41]. Analysis of the antibody response in H, L lines, and interline hybrids of the five selections indicated that high responsiveness was incompletely dominant, to a variable extent, in selections I, II, III, and IV, whereas it was incompletely recessive in selection V. In all selections, the high or low effects of the selected alleles are not limited to the selection antigen but may also influence the immune response to immunogens noncrossreactive with the selection antigen. This multispecific effect is not general, since the difference in antibody response between H and L responder mice to various unrelated antigens may be identical, smaller, insignificant, or, in a few instances, inverse as compared to the difference in response to the selection antigen. Comparison of the results in the five selections (Table 1) indicates that the multispecific effect is large in selections I and III, intermediate in selections II and IV, and restricted in selection V.

Improvement of the effect of selection was obtained by assortative breeding from two foundation populations, F0H and F0L, each of which was produced by

Table 1
Immunogenetic parameters of five selections

Selection	Agglutinin response to	Maximum interline difference (log_2 titer)	Selection limit	Heritability (h^2)	Number of loci	Multispecific effect
I	Sheep or pigeon RBC	7.8	F_{16}	0.21	9–15	Large
II	Sheep RBC	6.7	F_{13}	0.20	2–12	Intermediate
III	Salmonellae f	6.3	F_{16}	0.18	7–18	Large
IV	Salmonellae s	6.5	F_{13}	0.18	5–12	Large, nul, reversed
V	BSA or RGG	9.8	F_7	0.22	2–4	Small

balanced frequency of the gene pools from the H or 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 the selection was generalized to several antigens. These results obtained in selection GP for general-primary and selection GS for general-secondary 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 [42].

Selective breeding was also carried out for mitotic responsiveness of lymph node cells to in vitro stimulation by PHA. The selection limit was reached after 10 generations, the realized heritability was 0.24, the low responsiveness was incompletely dominant, and the character was under the control of 10–19 independent loci. These H and L responder mice also displayed a similar difference in responsiveness to Con A, in MLR, and graft versus host reaction (GvHR) but produced the same antibody titer when immunized with SRBC [43].

The Biozzi experimental approach of bidirectional selective breeding has also been used to investigate the polygenic control of chemical carcinogenesis. The selected character was the susceptibility and resistance to two-stage skin carcinogenesis, initiated with 7,12-dimethyl-1,2-benzanthracene (DMBA) and promoted with 12-0-tetradecanoyl-phorbol-13-acetate (TPA). The selection was started from a heterogeneous population as produced by the intercrossing of eight inbred strains of mice and the character chosen for the assortative mating was the number of skin papillomas at the end of the promotion period. Susceptible (Car-S) and resistant (Car-R) lines of mice separated by 10 consecutive generations of bidirectional selective breeding display a very large difference in responsiveness to carcinogenesis. The susceptibility of papilloma induction in interline F1 hybrids is an incompletely dominant character. The difference between Car-S and CarR lines is restricted to the skin implying that the selected genes produce a tissue-specific effect. No difference was found in antibody and cell-mediated responses between Car-S and Car-R mice [44].

3.2. Life span and tumor incidence in Biozzi mice

Whether selective breeding for a polygenic character, such as antibody responsiveness, also affects life span was investigated in selections I, II, and III. The life span was longer in H than in L mice of selections I and II [45,46], but no difference was found between H and L mice of selections III [47]. 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 [46]. Moreover, the life span of the last 20% survivors, which are 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. Noteworthily, the long life span was incompletely dominant in the total population, but was longer and completely dominant in 20% of the survivors (Table 2). Thus, the results of this analysis suggest that antibody

Table 2
Immune response and life span in mice from selection II

Mouse population	No. of mice	Log_2 agglutinin titer (mean ± S.D.)	Mean life span (days ± S.D.)	
			Total population	Last 20% survivors
H	131	11.4 ± 0.8	712 ± 148	852 ± 64
L	119	5.2 ± 1.0	446 ± 110	544 ± 74
F1	153	9.4 ± 0.7	649 ± 186	847 ± 62
F2	191	9.8 ± 1.2	614 ± 183	781 ± 85
BcH	174	10.8 ± 0.9	630 ± 215	848 ± 71
BcL	102	7.5 ± 1.4	564 ± 158	708 ± 100

responsiveness and life span are polygenic traits regulated by a small number of the same or closely linked loci. In these studies, the incidence of spontaneous malignant lymphomas was found markedly higher in L than in H mice of selections I and II, whereas no difference was found between L and H mice of selection III.

The influence of immune responsiveness on life span and tumor incidence was also investigated in H and L mice of the GS selection at the F16 generation [48]. It was found that the cumulative mortality rate is remarkably higher in L than in H mice, the difference being accounted for mostly by malignant lymphomas that are the major cause of death in L mice. The interline difference in life span and lymphoma incidence in mice of the GS selection is much larger than that observed in mice of selections I and II (Table 3).

L mice of the PHA selection exhibited the same life span but higher incidence of lymphomas and solid tumors as compared to H mice [49], supporting the role of T-cell-mediated mechanisms in anti-tumor immunity. This concept of immunologic surveillance need not conflict with the observation that the incidence of solid tumors is the same or even higher in H as compared to L mice of selections I, II, III, and GS as life span shortening caused by malignant lymphomas may have prevented, to a large extent, the late appearance of solid tumors in L mice.

Table 3
Immune response, life span, and lymphoma incidence in H and L mice

Selection	Line	No. of mice	Log_2 agglutinin titer	Mean life span (days ± S.D.)	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

Table 4
Life span and incidence of inflammatory and degenerative diseases, and of neoplasms in male mice selected for susceptibility (Car-S) and resistance (Car-R) to skin chemical carcinogenesis at F10, and in male interline F1 hybrids

Lines	Car-S	Car-R	F1
No. of mice	202	148	120
Mean life span (days ± S.D.)	508 ± 182	677 ± 190	632 ± 219
No. of autopsied mice	192	139	111
No. of cases (%)			
Pneumonia	113 (59)	90 (65)	88 (79)
Nephrosclerosis	16 (8)	12 (9)	7(6)
Lymphoid neoplasms	10 (5)	12 (9)	5(4)
Solid tumors	12 (6)	38 (27)	19(17)

Car-S ≠ Car-R, $P < 0.00005$; Car-S ≠ F1, $P < 0.00005$; Car-R ≠ F1, $P < 0.10$.

The cellular modifications of the immune system brought about by selection have been previously described and summarized elsewhere [39]. Briefly, a major difference between selections I and II on the one hand and selections III and IV on the other hand concerns macrophages. In selections I and II macrophages of L mice show a more active antigen catabolism and less efficient antigen presentation than macrophages of H mice. Conversely, no differences in macrophage activities were found between H and L mice of selections III and IV. NK cell activity was found lower in L than in H mice of selection I but higher in L than in H mice of selections II and III. In selection I, B-cells exhibit higher rates of proliferation and differentiation in H than in L mice whereas there is no difference between H and L mice in T-cells involved in helper activity, skin graft rejection, GvHR, PHA mitotic response, and DTH. Thus, it is apparent that no consistent cellular changes have been identified for a coherent interpretation of the immunologic mechanisms affecting life span and tumor incidence in H and L mice. Studies on innate and acquired immmunity to infections in selection I have revealed that H mice are more resistant than L mice to extracellular pathogens (*Pneumococcus*, *Klebsielia*, *Trypanosoma*, and *Plasmodia*) which are sensitive to the consequence of antibody binding, whereas L mice are more resistant than H mice to intracellular pathogens (*Salmonella*, *Brucella*, *Yersinia*, *Mycobacteria*, and *Leishmania*) which are sensitive to the bactericidal activity of macrophages. Although this dicotomy, which may largely depend on the antithetic activities of immunoregulatory molecules, is evident in young mice it is difficult to define the role of macrophages and infections in conditioning the life span of H and L mice. However, the critical role of environmental factors (viruses) is supported by the observation of maternal effects for life span and lymphoma incidence in mice of the GS selection [48].

The influence of loci controlling resistance and sensitivity to chemical carcinogenesis on life span and diseases has been examined in Car-R and Car-S mice that display no difference in antibody and cell-mediated immunity. Results obtained after 10 generations of selection (Table 4) show that the life span is shorter in Car-S

mice as compared to Car-R mice. Since there is no difference in life span between Car-R mice and the interline F1 hybrids, longevity appears as a dominant character. It is noteworthy that the percent of diseases is the same in Car-S, Car-R and their hybrids. Thus, selection of genes for resistance to chemical carcinogenesis has also selected genes for longevity, the former genes being recessive while the latter genes being dominant in F1 hybrids. Genetic segregation experiments will show whether tumor resistance and longevity are controlled by different genes.

4. Concluding remarks

The use of the Biozzi mice as animal model has provided the demonstration of a few genes that regulate immune responses and also affect life span and tumor incidence. This remarkable result was found in selections I, II and GS, but not in selection III, although in all cases the selected character was the antibody response. The difference in macrophage activity between H and L mice, as detected in selections I and II but not in selection III, may account for the different effects of these selections on both life span and tumor incidence. It is noteworthy that in the PHA selection the tumor incidence was higher in L than in H mice but no difference was found in life span. Thus, the role of T-cell activity appears very relevant for anti-tumor immunity but negligible for life span. Although different cellular components of the immune system may influence tumor incidence and life span, it is as yet unclear how genetic selection, e.g. against antibody responsiveness, brings about an increased incidence of malignant lymphomas that are the predominant cause of death. It would be interesting to examine whether the lymphoma incidence could be decreased and the life span be 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 from immune disfunctions.

The effects of genetic selection on the cellular components of the immune system are poorly defined and should be better evaluated not only in young but also in aging mice to assess whether the selected genes protect from the age-related cellular changes or further deteriorate the aging immune system. To this end, it would be desirable to breed also unselected control mice and to determine the effects of selection also in a specific-pathogen-free environment.

The study of mice selected for resistance or sensitivity to skin carcinogenesis has uncovered loci that control both resistance to tumor induction and longevity, while have no effects on immunity and disease incidence. Whether resistance and longevity are controlled by the same genes with pleiotropic effects or by two independent sets of genes may be tested in segregrating backcross populations. Since the genes regulating resistance or sensitivity to tumor induction are expressed in the skin, it would be interesting to look for changes in skin elasticity associated with resistance or sensitivity as a biomarker of aging. Loss of skin elasticity due to increased collagen cross-linking has, indeed, been considered a representative sign of aging [50].

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