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T Cell Subset Patterns That Predict Resistance to Spontaneous Lymphoma, Mammary Adenocarcinoma, and Fibrosarcoma in Mice

Richard A. Miller and Clarence Chrip

Aging leads to changes in the proportion of several T cell subsets in peripheral blood, but it is not yet known whether these changes have prognostic significance for late-life diseases. To examine this question, levels of T cell subsets were measured at 8 and 18 mo of age in the peripheral blood of mice of a genetically heterogeneous stock, and the mice were then subsequently evaluated for life span and for cause of death. The results indicate that mice whose T cell subset patterns look like those of old mice tend to die at earlier ages, regardless of the specific cause of death. At 18 mo, 39% of the variance within the set of seven measured subsets could be combined statistically into a single number, whose correlation with individual subsets suggested that it could be interpreted as an index of immunological aging. T cell subset pattern, as represented by this index, was a predictor of life span in mice dying of lymphoma, fibrosarcoma, mammary adenocarcinoma, or of all other causes considered together. Even as early as 8 mo of age, T cell subset patterns are significant predictors of all three forms of cancer, although at this age the association is stronger in mated female mice than in virgin mice. These results support two controversial hypotheses, which are not mutually exclusive: 1) early immune senescence might predispose to early death from cancer and 2) differences in aging rate, as monitored by tests of immune status, might accelerate or decelerate a wide range of late life neoplastic diseases. The Journal of Immunology, 2002, 169:1619–1625.

The Journal of Immunology
access to laboratory chow and fresh water. To document the specific pathogen-free status of the colony, sentinel animals (not part of the test population) were exposed to pooled spent bedding and then examined for pinworms and for serological evidence of infection with Sendai virus, mycoplasma, or mouse coronavirus. Such testing was conducted quarterly and proved negative throughout the course of the experiment.

In some cages of female mice, a stud male mouse was introduced at ~8 wk of age to create a group of “mated females.” Litters were removed from these cages within the first week after birth, and the male was removed when the females reached 6 mo of age. Cages of virgin males, virgin females, and mated females were all housed within the same room. Husbandry practices were in accordance with American Association of Laboratory Animal Care and institutional guidelines.

Exclusion criteria

Cages in which fighting among males had led to serious wounding were culled from the experiment (~25% of male cages, all at ages before 12 mo). Among the 571 mice for which complete sets of T cell subset measures were available at either 8 or 18 mo of age, 50 were excluded because it was not possible to infer a single cause of death from the necropsy. The remaining 521 animals are the subject of this report.

T cell subsets

Blood samples were taken by tail vein puncture at ages 8 and 18 mo. Each sample was divided into aliquots for two-color assessment of T cell subsets using flow cytometry. The protocol has been described in detail elsewhere (16) and is summarized in Table I.

Necropsy

Mice were inspected at least daily. Mice suspected to be ill (because of weight loss, poor grooming, or visible tumor) were observed twice daily except on weekends. Mice judged by an experienced technician to be so severely ill that survival for more than a few additional days was unlikely were taken to the necropsy suite and humanely euthanized; this group made up 59% of the total. Mice found dead (41%) were also submitted for necropsy. The necropsy protocol has been described in detail elsewhere (16) and involves both gross inspection and histological examination of sections from 37 organs.

Statistical analyses: introduction

The overall goal of the analysis was to see whether T cell subset patterns were predictors of life span in groups of mice dying of different causes. The approach used had three stages.

The first stage was to combine the observed T cell subset values into a single number, called a first principal factor, that could serve as an overall indicator of the T cell subset pattern for each individual mouse. The starting data set contained measures of seven different T cell subsets for each mouse. The computer algorithm takes each subset value, multiplies it by a weight, and then adds all seven of these weighted values together to calculate a sum, called the first principal factor, for each individual mouse. (For technical reasons, the actual subset values are first normalized, i.e., expressed as the number of SDs above or below the mean value for the subset across the whole set of mice.) The weights used are selected by the algorithm, so that the variation (SD) of the factor, within the whole group of mice, is as high as possible. This procedure assigns one new number, the principal factor, to each mouse as a quantitative measure of its T cell subset pattern. The PC algorithm, implemented in NCSS statistical software (NCSS Statistical Software, Kaysville, UT), calculates PC factors directly from the data (i.e., not from a correlation or covariance matrix) using multivariate regression methods.

Second, it is possible to calculate second and third principal factors to sum up variation among the mice that remains after adjustment for the first principal factor. These second and third factors, if evaluated in 18-mo-old mice, would be called F2_18 and F3_18. As shown in Results, these second and third factors were found not to be good predictors of life span, and so information about them is not included in the tables that present the regression results of the analysis.

Lastly, some of the tables also report “factor loadings.” These are ordinary correlations between the principal factor and the T cell subsets used in the calculations. A high positive factor loading implies that mice with high values of the particular T cell subset tend to score high in the principal factor. A high negative factor loading implies that mice with high values of the particular T cell subset tend to have low scores for the principal factor. A factor loading that is close to zero implies that the T cell subset has little influence on the principal factor score.

Statistical analyses: technical methods

The initial data set consisted of measures of seven T cell subsets for each of 521 mice, for which each T cell subset had been measured at 8 mo, at 18 mo, or at both ages. In previous work (17) we have found that four of these subsets, measured at 18 mo of age, were each individually a significant predictor of longevity in these four-way-cross mice. A PC method was used to combine data from these seven T cell subsets into a smaller number of composite indices to see whether one or more of the uncorrelated composite factors could serve as a predictor of remaining longevity. The PC algorithm, implemented in NCSS statistical software (NCSS Statistical Software, Kaysville, UT), calculates PC factors directly from the data (i.e., not from a correlation or covariance matrix) using multivariate regression methods. In each case the subset measurements were used as predictor variables: each of the seven PC factors with each of the original T cell subset measures was included in the calculation of the PC factor. These second and third factors, if evaluated in 18-mo-old mice, would be called F2_18 and F3_18. As shown in Results, these second and third factors were found not to be good predictors of life span, and so information about them is not included in the tables that present the regression results of the analysis.

The second stage of the analysis is to test the hypothesis that the first principal factor, calculated as explained above, can predict life span, considering the entire mouse population. The method used for testing this hypothesis is similar to linear regression, using equations in which one or several independent variables are used to calculate the expected value of a dependent variable. In this case, the dependent variable is a measure of the mortality risk, and the independent variables are the principal factor (the measure of T cell subset pattern, calculated as described above) plus other variables such as age and gender. The null hypothesis is that there is no relationship between T cell subset pattern and life expectancy. The regression calculation yields a $p$ value, the probability that the null hypothesis is correct. A low $p$ value provides support for the conclusion that T cell subset patterns, as condensed into a single principal factor, are indeed able to predict mortality risk and life span. The regression calculation also produces a regression coefficient that tells how strongly mortality risk is associated with the immune subset principal factor. For example, a coefficient of 1.37 would imply that a one-SD shift in the principal factor is associated with a 37% increase (or decrease) in mortality risk.

The third stage of the analysis is to repeat stage 2, but this time using only subpopulations of the mice, specifically groups of mice that differ by cause of death. The hypotheses to be tested here are that immune subset patterns are significant predictors of mortality risk in mice dying of lymphoma, in mice dying of fibrosarcoma, etc.

Three other points deserve note. First, the first principal factor can be calculated at any age. We use the term “F1_18” to refer to the first principal factor calculated using T cell subset data from mice 18 mo of age. “F1_8” refers to the same calculation, but using data from mice 8 mo of age. Second, it is possible to calculate second and third principal factors to sum up variation among the mice that remains after adjustment for the first principal factor. These second and third factors, if evaluated in 18-mo-old mice, would be calculated F2_18 and F3_18. As shown in Results, these second and third factors were found not to be good predictors of life span, and so information about them is not included in the tables that present the regression results of the analysis.

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Table I. T cell subsets examined

<table>
<thead>
<tr>
<th>Subset</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>% of CD3</td>
<td>CD3+, CD4+, helper T cells</td>
</tr>
<tr>
<td>CD8</td>
<td>% of CD3</td>
<td>CD3+, CD8+, killer T cells</td>
</tr>
<tr>
<td>CD4M</td>
<td>% of CD4</td>
<td>CD4+, CD4+&lt;sup&gt;high&lt;/sup&gt; memory CD4 cells</td>
</tr>
<tr>
<td>CD8M</td>
<td>% of CD8</td>
<td>CD8+, CD8+&lt;sup&gt;high&lt;/sup&gt; memory CD8 cells</td>
</tr>
<tr>
<td>CD4P</td>
<td>% of CD4</td>
<td>CD4+, CD4&lt;sup&gt;58&lt;/sup&gt; naive CD4 cells</td>
</tr>
<tr>
<td>CD8P</td>
<td>% of CD8</td>
<td>CD8+ cells expressing P-glycoprotein</td>
</tr>
</tbody>
</table>
Results

The pathologist (Dr. C. Chrisp) attempted to infer a most likely cause of death in each of the 571 cases that came to necropsy and in 521 of the cases was able to attribute the death or terminal illness to a specific diagnosis. A cause of death could not be assigned for 50 of the mice, either because of advanced autolysis or because there were several serious conditions that seemed likely to contribute to the lethal illness. Table II summarizes the results of this set of necropsies. Three groups of mice are shown that differ in gender and, for the females, in reproductive history. There are substantial differences among these three groups in the distribution of several varieties of neoplasia. Pituitary adenoma and lymphoma, for example, were more frequent as a cause of death for females than for males, whereas in contrast males tended to die more frequently of hepatic tumors or of pulmonary adenocarcinoma. The incidence of lethal mammary adenocarcinoma was more common in the mated than in the virgin females. Among males, 32% died of a mouse urinary syndrome (18, 19) that is thought to reflect psychological stress secondary to adjustments in dominance hierarchy among group-housed males. The miscellaneous group of nonneoplastic illnesses included hemangiosarcomas, a granulocytic leukemia, hardener gland adenocarcinomas, rhabdomyosarcomas, and squamous cell carcinomas, among other lesions. The miscellaneous group of nonneoplastic lesions included cases of endometritis, enamel organ dysplasia, glomerular amyloidosis, myocarditis, intussusception of the jejunum, and abdominal hematoma secondary to disseminated intravascular coagulation, among other lesions. Some form of neoplasia was deemed responsible for death in 82% of the diagnosable females and in 47% of the diagnosable males.

A PC analysis (PCA) was then performed to see whether the complexity in the T cell subsets database could be reduced to a smaller number of composite variables. Using T cell subset data derived from 8-mo-old mice, the calculation revealed three orthogonal factors with eigenvalues >1; thus each of these three composite variables captured more of the experimental variation than did the average subset within the panel of observed measurements. The first of these factors, with an eigenvalue of 2.01, accounted by itself for 29% of the variance in the set of observations. Table III includes the factor loadings for each of the three factors, referred to respectively as F1_8, F2_8, and F3_8. The factor loadings, which are the correlations between the factors and the individual T cell subset measures, show that high values of F1_8 are found in mice that, on average, tend to have relatively high levels of CD4M and CD8M cells and relatively low levels of CD4 and CD4V cells. Previous work in isogenic as well as in genetically heterogeneous mice (3) has shown that aging increases mean levels of CD4M and CD8M and decreases mean levels of CD4 and CD4V subsets, and these age effects were confirmed in the current set of mice (data not shown). Thus, high values of F1_8 identify mice whose immune systems resemble those of chronologically older mice, and in this sense F1_8 may be interpreted as a composite index of immunological aging. Being able to calculate for each mouse a single value that combines information from several T cell subsets into a numerical measure of T cell aging is a first step in testing the hypothesis that T cell changes may be associated with differences in late life susceptibility to diseases (see below).

Table II. Synopsis of necropsy outcomes

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Mated females</th>
<th>Virgin females</th>
<th>Virgin males</th>
<th>All mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestive heart failure</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Endometrial sarcoma</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>10</td>
<td>18</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Hepatocarcinoma</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>18</td>
<td>34</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Mammary adenocarcinoma</td>
<td>24</td>
<td>7</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Mouse urinary syndrome</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary adenocarcinoma</td>
<td>6</td>
<td>2</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Miscellaneous, non-neoplastic</td>
<td>13</td>
<td>15</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Miscellaneous, neoplastic</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Diagnosable cases (no.)</td>
<td>268</td>
<td>136</td>
<td>117</td>
<td>521</td>
</tr>
<tr>
<td>Total cases (no.)</td>
<td>293</td>
<td>147</td>
<td>131</td>
<td>571</td>
</tr>
</tbody>
</table>

a Necropsy-based diagnoses (as percentage of the diagnosable cases) from the 521 mice (of 571 total) for which the pathologist (Dr. C. Chrisp) was able to infer a likely cause of death. An additional 12 mice were excluded because they did not produce technically acceptable values for all seven T cell subsets at either 8 or 18 mo of age and thus could not be used for the regression analysis that is the focus of this report.
and middle-aged mice may reflect differences among mice in the pace of aging or the effects of aging on T cell homeostasis.

In the second stage of the analysis, proportional hazard regression was used to determine whether one or more of these factors, measured at either age, was a significant predictor of life span. Using data obtained at 8 mo, the regression model incorporated five factors as potential predictors of mortality risk: the three factors generated by the PCA calculation and two others reflecting gender and reproductive history. Of these, only F1_8 had a significant association with longevity. The first line of Table IV shows the results of this regression analysis for all 428 mice for which F1_8 could be computed. The first two columns show the strength of the association between F1_8 and life span (B) and the SE for this estimate. Exp(B) represents the relative increase in risk associated with a change of one SD in F1_8, i.e., 17% in this instance. This means that a mouse with a value of 1.0 for F1_8 that is one SD above the average would have a 17% increase in mortality risk compared with the average mouse. Similarly, a mouse with an F1_8 score one SD below average would show a 17% lower mortality risk than average. The Z statistic and its associated probability, p(Z), estimate the likelihood that B = 0. In this case, F1_8 is seen to be significantly (p = 0.001) associated with differences in longevity among the mice, with high values of F1_8 predicting reduced longevity. This indicates that the relationship between high scores of F1_8 and high mortality risk is unlikely to have resulted by chance alone. Two R² values are included in Table IV. The first of these shows R² for F1_8 by itself; for all-cause mortality, R² = 2%, indicating that interanimal variation in F1_8 accounts for only ~2% of the differences in mortality risk among these mice. The second R² shows the strength of the correlation using the entire model, i.e., including variance accounted for by the other two immunological factors, gender, and reproductive history. Thus, differences among mice in F1_8 account for a small, but significant, proportion of variation in life expectancy at this age.

In the third stage of the analysis, we determined whether F1_8 was a significant predictor of life span in groups of mice that had died of one of the three diagnoses for which we had at least 40 cases, i.e., lymphoma, mammary adenocarcinoma, or fibrosarcoma. The method was the same as that used for the first line in Table IV, except that this time the calculations were performed only on groups of mice that had died of each specific illness. Table IV shows that F1_8 is indeed significantly associated with life span in each of these three classes of animals. Indeed, this factor accounts for 12% of the variance in life expectancy in mice dying of mammary adenocarcinoma or of fibrosarcoma (as indicated by the values in the R² column) and for 6% of the variance in the lymphoma deaths. For the former two diagnoses, a change of one SD in F1_8 is associated with a 41% increase in mortality risk, as indicated by the value of 1.41 in the Exp(B) column. Fig. 1 shows scatterplots illustrating the relationship between F1_8 and life span in each of these three groups of mice. Neither F2_8 nor F3_8 had a significant association with mortality risk in any of these diagnostic subsets, although there was a marginal association (Z = 1.89, p = 0.06) for F3_8 in mice destined to die of fibrosarcoma (data not shown).

Table V shows the results of a similar proportional hazards regression using subset data from 18-mo-old animals. The model...
included all three factors (F1_18, F2_18, and F3_18) as well as gender and mating status, but only F1_18 was found to have a significant association with life span for all-cause mortality for any of the three diagnostic groups. The results in Table V show that F1_18 is a highly significant predictor of subsequent life span, with p(\(Z\)) < 0.0001 for all-cause mortality. F1_18 also has significant ability to predict life span in four independent subpopulations of mice, i.e., those dying of lymphoma, of mammary tumors, or of fibrosarcoma, as well as those mice dying of any other cause. These differences in T cell subset patterns account for 12–13% of the variance in life expectancy in the lymphoma and fibrosarcoma groups. Fig. 2 presents the scatterplots for the three leading causes of death.

These results established that the composite index of T cell subset pattern was a significant predictor of life span at both 8 and 18 mo of age when evaluated in the entire mouse population, as well as in subpopulations of animals dying of lymphoma, fibrosarcoma, or mammary adenocarcinoma. Because the tested population was heterogeneous in gender and mating history, we were able to evaluate a secondary hypothesis, specifically that the association between subset pattern and disease risk would be equally strong regardless of these two variables. To do this, we conducted analyses, similar to that shown for all-cause mortality in Tables IV and V, for three groups of animals: virgin males, virgin females, and mated females. Table VI shows the results. High values of Z are associated with low p values and correspond to a strong association between subset pattern and mortality risk for the indicated group of mice. For mice tested at 18 mo the association between F1_18 scores and life span was seen in all three groups of mice, indicating that at this age T cell subsets are good predictors of life span in virgin males, virgin females, and mated females. In contrast, only the mated female group showed a clear association between T cell status and life span when the T cell tests were made at 8 mo of age.

Lastly, to see whether high scores on F1_8 identify mice likely to have, later in life, high scores in F1_18, we examined the correlation between these two measures of immune status for the 366 mice for which we could calculate scores for both factors. These factors were indeed strongly correlated (\(R = 0.47, p < 0.0001\)) when all of the mice are considered together. Stratification by gender and mating history, however, showed that the association was strong in mated females (\(R = 0.59, p < 0.0001\)), modest in virgin females (\(R = 0.40, p = 0.001\)), and negligible in virgin males (\(R = 0.07, p = 0.61\)).

**Discussion**
Our results show that much of the variation among mice in T cell subset patterns reflects correlated change in age-sensitive T cell subsets and furthermore that a linear, composite combination of these subset values is a significant predictor of longevity among

**Table V. Summary of proportional hazards regression analysis predicting life span from immune subset values tested at 18 mo of age**

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>B</th>
<th>SE(B)</th>
<th>Exp(B)</th>
<th>Z</th>
<th>(p(Z))</th>
<th>(R^2) for Factor</th>
<th>(R^2) for Model</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>All causes</td>
<td>0.26</td>
<td>0.05</td>
<td>1.20</td>
<td>5.36</td>
<td>0.0000</td>
<td>0.06</td>
<td>0.06</td>
<td>458</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0.47</td>
<td>0.13</td>
<td>1.61</td>
<td>3.67</td>
<td>0.0002</td>
<td>0.13</td>
<td>0.18</td>
<td>83</td>
</tr>
<tr>
<td>Mammary adenocarcinoma</td>
<td>0.26</td>
<td>0.13</td>
<td>1.20</td>
<td>2.00</td>
<td>0.045</td>
<td>0.07</td>
<td>0.09</td>
<td>62</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>0.36</td>
<td>0.14</td>
<td>1.43</td>
<td>2.54</td>
<td>0.011</td>
<td>0.12</td>
<td>0.13</td>
<td>53</td>
</tr>
<tr>
<td>All others</td>
<td>0.20</td>
<td>0.07</td>
<td>1.22</td>
<td>2.69</td>
<td>0.007</td>
<td>0.03</td>
<td>0.03</td>
<td>260</td>
</tr>
</tbody>
</table>

The proportional hazards regression-modeled life span as a function of five predictor variables: F1_18, F2_18, F3_18, sex, and mating status. The parameters and significance tests shown in the table represent the association of F1_18 with the life span outcome, because none of the other four predictor variables had a significant (p < 0.05) effect on longevity in these analyses. The regression was calculated five times, once for all 458 eligible mice and then again for three subpopulations of mice dying of specific neoplastic diseases, and for all other mice as well. B indicates the slope parameter for F1_18 in the regression equation, and SE(B) gives its associated SE. Exp(B) reflects the relative increase in risk associated with a 1-U (i.e. one SD) change in F1_18. Z and \(p(Z)\) test the hypothesis that B = 0; thus a \(p(Z) < 0.05\) indicates that, for this set of mice, F1_18 is a significant predictor of life expectancy at age 18 mo. \(R^2\) for factor reflects proportion of life span variance explained by F1_18 in the regression. \(R^2\) for model indicates the proportion of life span variance explained by the entire set of five tested predictor variables.
fluences other than aging per se. The PC approach, by combining information from multiple, mutually correlated age-sensitive traits, may generate a more robust and reliable index of interindividual differences in aging rate. Any individual assay, for example a test of a specific T cell subset in a single blood sample, is likely to have a good deal of uncertainty, but the combination of results from related tests may increase the signal-to-noise ratio and thus provide stronger predictive power than any single assay by itself. The development of such age-sensitive indices in a genetically heterogeneous stock should help avoid the complications of strain-specific idiosyncrasies that have bedeviled previous attempts to seek a useful surrogate for biological age (20).

Although previous data from this laboratory have already shown that CD4, CD4 M, CD8 M, and CD4V subsets, measured in 18-mo-old mice, are significant predictors of all cause mortality (17), the newly available data on cause of death pathology, together with the PC method of analysis, lead to several new conclusions of interest. 1) The results in Tables IV and V show that the association between subset pattern and longevity is significant regardless of cause of death, at least for those illnesses that are common enough to provide reasonable statistical power. The data thus imply, for the first time, that three dissimilar forms of cancer (lymph, breast, and fibroblast) are influenced by the same underlying factors and furthermore that these common factors affect T cell subset levels by slowing or speeding the effects of aging on T cell balance. 2) By testing the subset/longevity association in four non-overlapping groups of animals (those dying of lymphoma, adenocarcinoma, fibrosarcoma, and, for the 18-mo-old mice, all other causes combined), the current study shows that the ability of T cell subset patterns to predict life span is not merely due to effects of a specific disease on T cells, or vice versa. 3) The individual T cell subsets, analyzed one at a time in the earlier paper (17), did not predict life span when measured at 8 mo of age. In contrast, the use of the PC method to “pool” data from different subset tests on the same mouse shows that the first PC is a significant predictor of life span (at least in mated females) even at this early age. This observation suggests that whatever developmental or pathological pathways link subsets to death, these are detectable in relatively young mice and thus do not reflect changes that occur only in old age, at least in mated females. The specific hormonal factors that produce the association between T cell subsets and longevity earlier in the life of mated female mice than in the other groups clearly deserve further experimental study.

We have not attempted in this paper to test the hypothesis that T cell subset patterns can predict the cause of death, to predict, for example, whether an individual will die of lymphoma, mammary cancer, or some form of nonneoplastic illness. The analytical strategy instead addresses a separate issue: can our composite index of immune function predict longevity, and can it do so for subgroups of mice destined to die of different specific illnesses? Our findings suggest that indeed a statistical combination of data from different T cell subset tests can predict longevity and thus reflects biological processes that influence the incidence or progression of late-life diseases, including important forms of cancer. The demonstration that the composite factors predict longevity in separate subpopulations of mice (i.e., those dying of different diseases) provides multiple independent replications of the association between factor value and longevity, thus greatly strengthening the inference that the association is not merely a statistical fluke.

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Mated Females</th>
<th>Virgin Females</th>
<th>Virgin Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (F1_8)</td>
<td>$Z = 4.41, p &lt; 0.0001, N = 259$</td>
<td>$Z = 0.44, p = 0.66, N = 99$</td>
<td>$Z = 0.09, p = 0.93, N = 70$</td>
</tr>
<tr>
<td>18 (F1_18)</td>
<td>$Z = 4.23, p &lt; 0.0001, N = 248$</td>
<td>$Z = 2.39, p = 0.02, N = 129$</td>
<td>$Z = 2.80, p = 0.005, N = 83$</td>
</tr>
</tbody>
</table>

* This table shows the results of proportional hazards regression analyses, similar to those summarized in Tables IV and V, but for subpopulations of mice that differ in their gender and mating history. The first row shows the results of tests for the ability of F1_8 to predict life expectancy in mated females, virgin females, and virgin males. In each case the $p$ value is the chance that the indicated $Z$ statistic would result by chance alone.
These correlations we have documented are consistent with two theoretical models. The “immune protection” model would suggest that the effect of aging on the immune system creates a characteristic pattern of T cell subset changes that can speed up or slow down multiple forms of neoplasia. It is difficult to disprove this idea, but there are at present no compelling reasons to think that tumor incidence or progression would be influenced by the relative proportions of CD4 M, CD8 M, CD4P, CD4, or CD4V cells, and there is a substantial body of evidence suggesting that alterations in immune function are not themselves a major risk factor for the forms of neoplasm most common in older individuals. It also seems unlikely that alterations in immune status detectable in 8-mo-old mice would influence the pace of diseases that are typically not detectable until 18–24 mo of age and that typically do not cause death until 27 mo of age. The varieties of cancer seen most commonly in our mice—lymphoma, mammary adenocarcinoma, and fibrosarcoma—overlap only partially with those common among older humans, but do reflect substantial diversity in germ layer and tissue origin, rate of progression, and viral or nonviral etiology. This diversity also argues against the “immune protection” model, because it seems unlikely that alterations in protective immunity would have similar effects on the timing of such biologically disparate forms of neoplasia, representing in this case tumors of hematopoietic, connective tissue, and secretory cell origin.

A second model suggests that both increased mortality risk and altered T cell subset patterns are dependent on interindividual differences in biological aging rate. This “gerontological” model implies that just as members of different species age at different rates, so might individual mice differ in the rates at which they exhibit changes in age-dependent traits, including changes both in age-sensitive T cell subset levels and in those unknown factors that increase the risk of late-life illness. It is particularly noteworthy that these composite immune indices are significant predictors of life span in subsets of mice dying of different causes, i.e., lymphoma, fibrosarcoma, and mammary adenocarcinoma, because such a finding suggests that the incidence or progression of all three forms of illness may be timed by a common pacemaker. The evidence that F1_18 is also a significant predictor of longevity in the group of mice dying of causes other than the three listed tumors suggests that one or more other late-life illnesses may also be regulated by this pacemaker. The gerontological model makes the prediction that middle-aged mice with relatively extreme levels of an age-sensitive immune factor (such as F1_18) will also show relatively extreme values in tests of other age-sensitive traits, such as changes in bone structure, muscle function, and patterns of gene expression. We have presented elsewhere (15) preliminary evidence that middle-aged mice with relatively advanced T cell subset aging also show relatively weak muscle strength, and a more comprehensive set of similar studies is now under way. Some of the age-sensitive T cell subsets used in the PCA calculation are known to be under the control of polymorphic alleles in this stock of genetically heterogeneous mice (21), and it will be interesting to determine to what extent the three immune composite variables may be regulated by segregating alleles.

The stratification by gender and mating history (Table VI) suggests that the association between immune status and life expectancy may develop more rapidly in mated female mice than in virgin males or virgin females, although it is apparent in all three subpopulations by 18 mo of age. A previous analysis of individual T cell subsets (17) has shown that the relative ability of specific T cell subsets to predict longevity differs between mated and virgin females and also between virgin males and virgin females. The PC approach has allowed us for the first time to calculate an index of immunological age that predicts longevity in all three of these populations, does so for multiple causes of death, and has prognostic power as early as 8 mo of age.

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References