Hematopoiesis in the Aged: A Model of Hematopoietic Dysregulation?

By Gerald Rothstein

HEMATOPOIESIS IN THE AGED: A MODEL OF HEMATOPOIETIC DYSREGULATION?

In recent years, data have accumulated which establish that at least in some subsets of aged laboratory animals and humans, the pool sizes, function, and proliferation of circulating blood and marrow cells differ from those of young adults. In this issue of Blood, Sansoni et al make a further contribution by defining the populations and cytotoxic activity of circulating lymphocytes in apparently healthy people, from 4 to 106 years of age. In doing so, the investigators contribute convincing new information regarding the numbers of freely circulating lymphocytes, their subtypes, and their function extending from childhood into extreme old age. The authors’ meticulous attention to the health status of subjects and the display of individual data elements give a particular clarity and interest to the study, and these approaches are important ones for others to follow in investigating the biology of aging. Furthermore, the data for centenarians are a basis for speculation regarding the fundamental processes that underly the biologic changes which occur during human aging.

The investigators show that the number of circulating CD4+, CD8+, and B cells decreases during aging, but the number of activated T cells increases. Natural and redirected killer (NK and RDK) activity were similar when the youngest subjects (age 19 to 36) were compared with the centenarians. However, subjects with a mean age of 63.5 years had lower NK activity than either the young adults or centenarians. These results confirm and add to previous observations by Ligthart et al,2 who reported that carefully screened apparently healthy elderly individuals (age 75 to 84 years) had fewer circulating T (CD3+) cells than subjects age 25 to 34. Ligthart did not observe the reduction in CD4+ and B cells that was found by Sansoni et al, but this may be explained by the Sansoni’s analysis of a minimum of 10,000 cells/individual by automated techniques, rather than the much smaller sample sizes with the microscopic analyses used by Ligthart.

Sansoni et al also report a reduction in the natural killer (NK) activity of cells from individuals whose average age was in the mid 60s, but not in centenarians. These results are expressed on the basis of standardized numbers of total light density mononuclear cells, and were not adjusted for their content of cells known to mediate non-major histocompatibility complex restricted cytotoxicity, such as CD16+, CD56+, or CD57+. Because centenarians did exhibit greater circulating numbers of such cells, an adjustment for this might have also disclosed a significant defect in this extremely old population. Interestingly, in subjects with a mean age of 80, also subjected to very rigorous health screening, Ligthart et al did not find a defect in NK activity.3 At first glance, Sansoni’s finding of a cytotoxic defect only in the group in their sixth and seventh decades might be taken to indicate that the NK activity is impaired during the sixth and seventh decades, but that later on during aging, the defect is restored. However, another and perhaps more attractive hypothesis is that in those who ultimately survive into their 11th decade, the usual physiologic consequences of aging may be minimized or reduced in magnitude throughout life. Indeed, it can be postulated that the longevity of centenarians may be contributed to by their relative freedom from usual adverse age-associated changes in physiology. If this is correct, observations for the middle group studied by Sansoni et al (mean age 63.5 years) may be the most representative of lymphocyte physiology during usual aging.

The present study provides convincing evidence for an age-associated decrease in circulating CD4+ and CD8+ T cells and CD19+ B cells in healthy individuals during aging. However, age-associated changes in circulating NK cells and total NK activity have been only variably observed by other investigators. Whether a decline in NK activity is a regular occurrence in the healthy elderly seems doubtful, based on numerous reports of its absence. However, in might be proposed that NK activity is normal in the elderly in the absence of disease, and that in the instances in which NK activity is reduced, unrecognized illness in study subjects may have accounted for it.

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THE CONCEPT OF AGING AS A PHENOTYPE

A major consideration for all studies of the biology of aging is that presently, aging can only be quantified chronologically; there is no biologic marker for aging. Even so, considerable effort has been expended in attempting to identify a unifying mechanism that accounts for the entire range of the phenotype of aging in all organ systems. This is a meritorious aim, but it seems unlikely that the biology of aging will yield up its secrets in so direct a fashion. Another strategy is to first determine the characteristics of aging, a phenotype of aging, to purify the phenotype by separating out elements that appear to be conventional well-known disease processes, and then to seek unifying mechanisms to explain broad elements of the purified phenotype. In recent years, such a phenotype of aging and the hematopoietic system evolved, and the study of Sansoni et al contributes significantly to it. Next, studies will be needed to address the physiologic relevance of these reported changes in the lymphocytes. For example, it is not clear whether the age-associated decrease in the circulating pool of lymphocyte subtypes reflects a decrease in the total body lymphocyte mass. It will also be of interest to identify the underlying cause of T-cell activation, to explore the physiologic significance of the reduced NK activity in the 50 to 68-year-old group, and to determine whether the alterations in circulating lymphocytes reflect a change in lymphopoiesis or redistribution of the total lymphocyte mass.

INTERPRETING AGE-ASSOCIATED CHANGES IN HEMATOPOIESIS

The study of Sansoni et al highlights at least one critical element for interpreting studies of aged humans, the issue of health status. The necessity of such screening has been clearly demonstrated in the work of Ligthart et al, who found that the number of circulating lymphocytes, CD4+ cells, and B cells of elderly individuals and young adults did not differ if study subjects were meticulously examined and found to be healthy. In contrast, elderly individuals who did exhibit abnormalities in their standard complete blood counts or blood chemistries also exhibited significant reductions in each of these cellular populations. Furthermore, small or insignificant differences that had existed between the age groups of healthy individuals were greatly magnified by studying the elderly subjects who failed health screening, even if they were compared with unwell young subjects. Thus, abnormalities in the screening examination were associated with alterations in the elderly subjects which did not occur in the young group. Previous studies are consistent with the notion that strict criteria for establishing health status are also necessary for the study of hematopoiesis in elderly subjects. An example is provided by the study of Lipschitz et al, who performed ferrokinetic measurements of marrow mass in either carefully examined healthy young and elderly adults, or in elderly adults who were deemed not healthy due to unexplained anemia. In the marrow of the healthy nonanemic elderly subjects, hematopoiesis appeared normal, and only the pool size of total myeloid precursors was smaller than that of young controls. However, in anemic elderly subjects, the pool of marrow normoblasts was paradoxically small, and the numbers of the progenitors, CFU-E and CFU-C, were significantly reduced. In the peripheral blood, reductions in blood neutrophil and lymphocyte counts were also observed. Studies of aged mice show similar observations to those in humans: normal marrow mass and hematopoiesis in the unperturbed steady state, but disordered hematopoiesis during increased hematopoietic demand. For example, numerous studies have established that the total mass of marrow in old C57BI mice is equal to or greater than that of young adult animals. However, when healthy young or aged mice were bacterially challenged with sublethal doses of Escherichia coli, the neutrophil storage pool of young mice was only partially used and then its cellular population was rapidly replaced. In contrast, the neutrophil storage pool of bacterially challenged aged mice was much more depleted and then failed to regenerate. When progenitors and their proliferation were examined, the bacterially challenged young mice responded to increased hematopoietic demand with an appropriate increase the number and turnover of the marrow’s colony-forming unit granulocyte macrophage (CFU-GM), whereas aged animals experienced a paradoxical decrease in the number and turnover of CFU-GM. A similarly impaired regenerative response was noted by Boggs and Patrone, who found that phlebotomized aged mice exhibited an impaired post-phlebotomy restoration of red blood cell mass to pre-phlebotomy levels.

SIGNIFICANCE OF VARIATIONS IN THE EXPRESSION OF THE AGE-ASSOCIATED PHENOTYPE OF HEMATOPOIESIS

Based on the above studies and other observations, an age-associated phenotype of hematopoiesis can be identified in laboratory animals and humans. The characteristics of the phenotype are: in healthy elderly subjects, steady-state hematopoiesis is not demonstrably impaired, but in response to such perturbations as bacterial infection or during periods of increased hematopoietic demand, hematopoiesis becomes sluggish or even paradoxically downmodulated and a latent defect is thus expressed. This results in impairment of hematopoietic regeneration and difficulty in achieving new states of hematopoietic equilibrium. It can be postulated that this impairment in the hematopoietic response to increased demand may contribute to the increase in toxicity experienced by elderly subjects treated with cytotoxic chemotherapy, and some instances of the neutropenia observed in elderly subjects with bacteremic pneumococcal pneumonia. It has also been proposed that an age-associated impairment in hematopoiesis contributes to the increase in the incidence of unexplained anemia in the elderly. The latency of the age-associated defect might also explain the variability observed in the unmasking or accentuation of alterations in circulating lymphocyte pools and in the appearance of impaired functional properties, such as NK activity. In the present study of Sansoni et al, careful screening appears to have minimized unrecognized physiologic perturbations and therefore eliminated...
much of the age-associated changes that have been variably reported elsewhere. Paradoxically, however, restricting study to only healthy elderly subjects may actually be an impediment to recognizing and studying the age-associated alterations in hematopoiesis, because these changes may be maximally expressed in animals or humans who are not healthy, such as those with bacterial infection, uncompensated anemia, or malnutrition. Indeed, it seems likely that the age-associated phenotype becomes most prominent as a consequence of a two-hit process, with the latent defect of aging being enhanced in its expression due to intercurrent illness. It will likely be productive in the future to also conduct studies of blood cell pool sizes, distribution, production and function, intentionally comparing unwell aged and young animals or humans who are rigorously matched for disease and severity of illness. Those experiments, although admittedly challenging and complex, should produce important and illuminating results.

AGE-ASSOCIATED HEMATOPOIESIS:
A DYSREGULATED STATE?

The mechanism that is responsible for the age-associated changes in the cells of the blood and marrow has not been identified. However, a number of observations suggest that the phenotype is not likely caused by unresponsiveness of hematopoietic precursors to growth stimulators. In fact, accumulating evidence supports the concept that in vivo, the elderly’s progenitors are responsive to recombinant factors. Shank and Balducci reviewed the literature to compile the published responses of subjects younger or older than 65 years of age to GM colony-stimulating factor (GM-CSF), G-CSF, interleukin-3 (IL-3), and erythropoietin. No differences were found in the mean-fold increment in circulating granulocytes or in the posttreatment increment in hemoglobin concentration. Stein et al have also reported that age was not a determinant in the responsiveness of elderly subjects with myelodysplastic syndrome to erythropoietin. These reports represent relatively few observations, but support the hypothesis that growth factor responsiveness of hematopoietic progenitors is preserved during aging.

Other observations point to impaired or dysregulated production of cytokines as a candidate mechanism for the age-associated defect. For example, Lee et al have shown that IL-1-induced production of biologically active CSF by marrow stromal cells of the elderly is diminished. In other studies, peripheral blood mononuclear cells from healthy elderly subjects displayed reduced expression of GM-CSF when compared with those of young adults, but normal expression of other cytokines such as IL-3 and tumor necrosis factor. In aged mice, there is similarly reduced expression of GM-CSF by splenic cells, but normal expression of some other cytokines and overexpression of yet others, such as IL-6. Thus, in apparently healthy aged humans and laboratory animals, there is a consistent picture of impaired expression of GM-CSF, but there is not uniform impairment of expression for all other cytokines. These observations suggest that the ability of cells to complete the stimulus-response loop and produce cytokines is not universally impaired; the observations are more in keeping with the hypothesis that during aging, the expression of cytokines is dysregulated, and this dysregulation may contribute to or cause the age-associated hematopoietic phenotype. The concept of age-associated alterations in peripheral regulators, rather than the end organ itself, is supported by cross-transplantation studies in which aged or young cells assume the phenotype of the old or young animal into which they are deposited.

HEMATOPOIESIS AND AGING: AN EVOLVING PICTURE

During the past decade, the elements that constitute the phenotype of hematopoiesis during aging have been more clearly defined. The study of Sansoni et al is yet another significant contribution to that clarity as it applies to the lymphocytes, and future studies should be directed toward understanding the mechanisms that account for the phenotypic features, such as those described by these and other investigators. Detailed study of the expression of GM-CSF and other cytokines in the elderly may provide new insights into the regulation of expression for the genes from which they are encoded. In addition, such information may be useful in devising strategies for promoting a vigorous hematopoietic response in elderly subjects. In the course of such studies, more information also will be needed regarding the responsiveness of progenitors of aged subjects to growth factors, both in vitro and in vivo, and further exploration will be needed in the question of potential reversibility of the defect. The studies of the Daynes group, which show that treatment of aged animals with dehydroepiandrosterone sulfate (DHEAS) induces reversion of their pattern of cytokine expression to a younglike phenotype, should be extended to studies of elderly humans.

Degas, the great artist, was once asked why he painted pictures of ballerinas. He is said to have replied that it was because ballerinas were a good excuse for making paintings. The Degas position might also be applied to studies of hematopoiesis during aging. Indeed, the aging phenotype appears to be a unique opportunity for understanding more about the mechanisms responsible for a regularly occurring, if complex, disorder in the regulation of hematopoiesis.

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