Commentary

Molecular Alterations in the Aging Immune System

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The study of the developing immune system during fetal and early postnatal life has contributed substantially to the biological and molecular understanding of the immune response. At the other extreme of lifespan, the immune systems of aged animals and humans also undergo characteristic changes, often in the direction of apparently decreased immune competence. Aging-related changes have been widely recognized for some time, but many have defied cellular or molecular characterization. Recently, however, a number of findings have begun to elucidate the mechanisms underlying aging-related immune system changes.

It has been widely observed that, with aging, substantial changes occur in both the functional and phenotypic profiles of T lymphocytes in humans and in experimental animal models. These changes include a shift toward greater proportions of $CD4^+$ T cells of memory ($CD44^{hi}CD45RO^+$) phenotype and fewer cells of naive ($CD44^{lo}CD45RA^+$) phenotype (1–5). Paralleling these phenotypic changes are functional changes that occur with aging, including decreased proliferation of T cells in response to TCR- and costimulus-mediated stimulation (1–3, 5–7) and alterations in the profiles of cytokines produced in response to T cell activation (2, 3, 5, 8). Aging-related changes in the early signal transduction events that occur in response to T cell stimulation have also been described (9–11). It has been suggested that some, but perhaps not all, of these changes in T cell responsiveness are the result of shifts from naive to memory T cell predominance with age.

Zhou et al. (12) have pointed to a role for expression of fas in some changes that occur in T cells with age. Fas is a cell surface molecule that transduces signals which mediate apoptotic cell death (13). In their report, Zhou et al. demonstrate that expression of fas, as well as susceptibility to fas-mediated cell death, is decreased in thymocytes and peripheral T cells of aged mice. To analyze the effect of fas expression, these investigators introduced a *CD2-fas* transgene, which reversed the age-dependent loss of fas and achieved sustained fas expression by T cells. Moreover, these investigators observed that expression of the *fas* transgene prevented a number of age-related T cell changes, including thymic involution, the shift toward a memory ($CD44^{hi}$) phenotype, the decrease in T cell proliferative response to anti-CD3 stimulation, and the altered cytokine profile of activated T cells.

The mechanism by which fas mediates these effects is unclear. The authors observed that peripheral T cells in *fas* transgenic mice do not undergo the usual age-related decrease in susceptibility to fas-mediated apoptosis, consistent with the hypothesis that fas-mediated apoptosis normally leads to elimination of less functional cells and, therefore, to maintenance of a more functional T cell population through cellular renewal. However, an equally striking observation by Zhou et al. is that *fas* transgenic mice show decreased apoptosis of thymocytes in response to anti-CD3 cross-linking in vitro, suggesting that fas expression might actually protect some thymocyte subpopulations from apoptotic death. It will be of interest to further characterize the role of fas in the specific events that occur during T cell differentiation and in the course of immune responses, and to evaluate the effect of fas expression on other organ systems and on overall longevity. In addition, a critical issue recognized by Zhou et al. is the identification of the mechanism that underlies decreased fas expression with age. This issue is of interest both as a basic problem of gene regulation and expression, and in the context of its implications for intervening to prevent what may be the undesired consequences of diminished fas function.

Recent results have also provided insights into the mechanisms underlying other long-observed changes that occur in the immune responsiveness of aged organisms. The in vivo antibody responses of aged rodents to T helper–dependent (TD) antigens are reduced in both titer and affinity when compared to the responses of young animals (14). The antigens studied have included a number of model proteins, among them viral or bacterial antigens relevant to clinical immunization (14, 15). Using idiotypic markers (16, 17) or in situ hybridization with V_H- and V_L-specific probes (18, 19), it has recently been shown that the germline Ig repertoire used by B cells changes with age, although the mechanism(s) underlying these changes in V region usage have not been elucidated. The generation of high affinity TD Ab responses depends, not only on germline gene usage, but also on a unique mechanism of somatic hypermutation of the variable regions of heavy and light chain genes. Somatic mutation appears to occur during ongoing immune responses predominantly within germinal centers (GC), and is followed by the selective survival of those B cells that by chance express mutated Ig receptors with high affinity for the selecting antigen (20). Selective survival of B cells in this process appears to depend on a number of factors, including encounter of specific antigen- and CD40-mediated interactions with T cells. After immunization, aged mice develop GC that have normal histologic architecture. Recently, however, it has been shown that somatic mutation fails to occur in the GC of aged

mice, correlating with the failure of these mice to develop high affinity Abs (21). Moreover, it has been shown recently that the GC of aged mice, although morphologically normal, fail to express the B7-2 costimulatory molecules that are normally expressed in GC of young animals in response to TD antigen (22). The abnormalities in Ab responses of aged animals thus have molecular correlates in reduced Ig gene somatic hypermutation and in reduced expression of costimulatory ligands. Further genetic and biochemical comparisons of these events in young and aged animals may provide additional insights into the basic mechanisms of immune response as well as into age-specific changes.

A number of additional areas appear to be opportune for study of the mechanisms underlying age-related immune changes. Substantial progress has been made in the past few years in elucidating multiple pathways for signal transduction through the cell surface receptors of T and B lymphocytes, antigen-presenting cells, and NK cells. The altered response of cells from aged donors can therefore be analyzed with an increasingly powerful set of probes for these pathways, which should allow extension of recent efforts in this area. Regulation of cell cycle progression and cell senescence is a topic that has been central to basic biologic studies of aging. The application of this perspective to the aging immune system is an area that is likely to be eluminating as it relates to alterations in replicative potential of immune cells and the consequences for immune responsiveness.

In August 1994, the National Institute of Allergy and Infectious Disease and the National Institute on Aging jointly sponsored a meeting to assess the state of research on the immunobiology of aging. The published report of that meeting will provide additional perspectives on this field and its current scientific opportunities (23).

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References

1. Lerner, A., T. Yamada, and R.A. Miller. 1989. Pgp-1^{hi} T lymphocytes accumulate with age in mice and respond poorly to concanavalin A. *Eur. J. Immunol.* 19:977–982.
2. Nagelkerken, L., A. Hertogh-Huijbregts, R. Dobber, and A. Dräger. 1991. Age-related changes in lymphokine production related to a decreased number of CD45RB^{hi} CD4$^+$ T cells. *Eur. J. Immunol.* 21:273–281.
3. Ernst, D.N., W.O. Weigle, D.J. Noonan, D.N. McQuitty, and M.V. Hobbs. 1993. The age-associated increase in IFN-γ synthesis by mouse CD8$^+$ T cells correlates with shifts in the frequencies of cell subsets defined by membrane CD44, CD45RB, 3G11, and MEL-14 expression. *J. Immunol.* 151:575–587.
4. Gabriel, H., B. Schmitt, and W. Kindermann. 1993. Age-related increase of CD45RO$^+$ lymphocytes in physically active adults. *Eur. J. Immunol.* 23:2704–2706.
5. Dobber, R., M. Tielemans, H.D. Weerd, and L. Nagelkerken. 1994. Mel14$^+$ CD4$^+$ T cells from aged mice display functional and phenotypic characteristics of memory cells. *Int. Immunol.* 6:1227–1234.
6. Song, L., Y.H. Kim, R.K. Chopra, J.J. Proust, J.E. Nagel, A.A. Nordin, and W.H. Adler. 1993. Age-related effects in T cell activation and proliferation. *Exp. Gerontol.* 28:313–321.
7. Engwerda, C.R., B.S. Handwerger, and B.S. Fox. 1994. Aged T cells are hyporesponsive to constimulation mediated by CD28. *J. Immunol.* 152:3740–3747.
8. Hobbs, V.M., W.O. Weigle, D.J. Noonan, B.E. Torbett, R.J. McEvilly, R.J. Koch, G.J. Cardenas, and D.N. Ernst. 1993. Patterns of cytokine gene expression by CD4$^+$ T cells from young and old mice. *J. Immunol.* 150:3602–3614.
9. Shi, J., and R.A. Miller. 1993. Differential tyrosine-specific protein phosphorylation in mouse T lymphocyte subsets: effect of age. *J. Immunol.* 151:730–739.
10. Whisler, R.L., and I.S. Grants. 1993. Age-related alterations in the activation and expression of phosphotyrosine kinase and protein kinase C (PKC) among human B cells. *Mech. Ageing Devel.* 71:31–46.
11. Utsuyama, M., Z. Varga, K. Fukami, Y. Homma, T. Takenawa, and K. Hirokawa. 1993. Influence of age on the signal transduction of T cells in mice. *Int. Immunol.* 5:1177–1182.
12. Zhou, T., C.K. Edwards, and J.D. Mountz. 1995. Prevention of age-related T cell apoptosis defect in CD2-*fas* transgenic mice. *J. Exp. Med.* 182:129–137.
13. Nagata, S., and P. Golstein. 1995. The fas death factor. *Science (Wash. DC).* 267:1449–1456.
14. Klinman, N. 1981. Antibody-specific immunoregulation and the immunodeficiency of aging. *J. Exp. Med.* 154:547–551.
15. Araneo, B.A., M.L. Woods II, and R.A. Daynes. 1993. Reversal of the immunosenescent phenotype by dehydroepiandrosterone: hormone treatment provides an adjuvant effect on the immunization of aged mice with recombinant hepatitis B surface antigen. *J. Infect. Dis.* 167:830–840.
16. Nicoletti, C., X. Yang, and J. Cerny. 1993. Repertoire diversity of antibody response to bacterial antigens in aged mice. III. Phosphorylcholine antibody from young and aged mice differ in structure and protective activity against infection with *Streptococcus pneumoniae*. *J. Immunol.* 150:543–549.
17. Gueret, R., A. Grandien, J. Andersson, A. Coutinho, J. Radl, and M.E. Weksler. 1993. Evidence for selective pressure in the

appearance of monoclonal immunoglobulins during aging: studies in M54 μ-transgenic mice. *Eur. J. Immunol.* 23:1735–1738.

18. Viale, A.-C., J.A.B. Chies, F. Huetz, E. Malenchere, M. Weksler, A.A. Freitas, and A. Coutinho. 1994. V_H-gene family dominance in ageing mice. *Scand. J. Immunol.* 39:184–188.
19. Yang, X., J. Stedra, and J. Cerny. 1994. Repertoire diversity of antibody response to bacterial antigens in aged mice. IV. Study of V_H and V_L gene utilization in splenic antibody foci by in situ hybridization. *J. Immunol.* 152:2214–2221.
20. Gearhart, P. J. 1993. Somatic mutation and affinity maturation. *In* Fundamental Immunology. 3rd ed. W.E. Paul, editor. Raven Press Ltd., New York. pp. 865–885.
21. Miller, C., and G. Kelsoe. 1995. Immunoglobulin hypermutation is absent in the germinal centers of aged mice. submitted for publication.
22. Miller, C., G. Kelsoe, and S. Hua. 1994. Lack of B7-2 expression in the germinal centers of aged mice. *Aging Immunol. Infect. Dis.* 5:249–252.
23. Task Force Report Report on Immunology and Aging: Prospects for Future Research. 1995. G.N. Abraham, A.M. McCormick, J. Albright, and R.A. Goldstein, editors. In press.