AGING AND ANTIMICROBIAL IMMUNITY

Lowered Efficiency of Protective T Cells as a Contributing Factor for the Decreased Resistance of Senescent Mice to Listeriosis

By PARSOTTAM J. PATEL

From the Trudeau Institute, Inc., Saranac Lake, New York 12983

We used infection of mice with Listeria monocytogenes as a model to study the effect of aging on antimicrobial immunity. The acute nature of this infection and the knowledge that acquired immunity to primary Listeria infection in mice is cell mediated in nature without any involvement of antibodies (1, 2) makes murine listeriosis one of the most suitable models to study the age effect on antimicrobial immunity and the effects of aging on cell-mediated immunity in general.

Using this mode, it was shown previously (3) that senescent mice (24 mo or more old) show markedly lower capacity to resist Listeria infection than young (8 mo old) mice. This decreased capacity to resist listeriosis was shown not to be caused by a deficiency in the innate mechanisms of antimicrobial resistance of senescent mice, but was the result, instead, of an age-related decline in the capacity to generate immunologically specific antibacterial immunity. Moreover, this was associated in senescent mice with a response that resulted in a smaller than normal increase in spleen weight, spleen cellularity, and splenic T cell content. This suboptimum response was associated with a profoundly decreased capacity of spleen cells obtained at the time of peak response from Listeria-immune senescent donors to adoptively immunize young syngeneic recipients against a lethal Listeria challenge infection (4). In this report, I present the results of a comparative dose-response analysis between anti-Listeria protective T cells from young and senescent donors. It will be shown that not only were there fewer protective T cells generated in response to immunizing infection of senescent mice with Listeria, but, on a per cell basis, protective cells from senescent donors showed at least 100-fold lower efficiency in protecting young syngeneic recipients than T cells from young donors.

Materials and Methods

Mice. AB6F1 (A/Tru × C57BL/6) mice of either sex were used. This is a long-lived hybrid with a median life expectancy of ~28 mo (5). All mice were bred and aged under specific pathogen-free conditions in the Trudeau Institute. Young mice used were 8–12 wk old, whereas senescent mice were 24–48 mo old. There were at least five mice in each group.

Bacteria. L. monocytogenes strain EGD was used. A large log phase culture (2 × 10⁶ bacteria/ml) was grown in Trypticase soy broth and frozen at −70°C in 1-ml portions. For each

* Supported by grant AG-01234 from the National Institute on Aging and Biomedical Research, U. S. Public Health Service; and grant 5501 RR05705 from the General Research Support Branch, Division of Research Resources, National Institutes of Health, Bethesda, Maryland.
experiment, a vial was thawed and diluted in saline to contain required numbers of bacteria in a volume of 0.2 ml for intravenous infusion. *Listeria* was enumerated by homogenizing spleens and livers in saline and plating 10-fold serial dilutions of whole organ homogenates on Trypticase soy agar.

**Adoptive Transfer of Anti-Listeria Immunity.** Spleen cell suspensions from normal and *Listeria*-infected donors were prepared as described (4). Cells were washed, counted, and resuspended at a desired density. Normal young (8–12 wk old) or senescent (24 mo old) recipients were infused with immune or normal spleen cells and challenged 1 h later with $5 \times 10^6$ *Listeria* intravenously. The organisms were enumerated 48 h later in the spleens of recipient mice. Protection was expressed as the log$_{10}$ difference between the number of *Listeria* in the spleens of recipients of immune spleen cells and normal control 48 h after the challenge infection.

**T Cell Enrichment.** Spleen cells were enriched for T cells by panning on anti-Ig-coated plastic petri dishes according to the procedure described in detail previously (4).

**Radio labeling of T Cell-enriched Spleen Cells.** Spleen cell suspensions were treated for 1 min with 0.83% ammonium chloride in Tris buffer (pH 7.2) to lyse erythrocytes. After washing, the cell suspensions were enriched for T cells by panning on anti-Ig-coated dishes, and the enriched T cells were labeled with sodium ($^{51}$Cr) chromate (CJSL, Amersham, Des Plaines, IL) or with $[^{3}H]$thymidine (25 Ci/mmol, New England Nuclear, Bethesda, MD). $^{51}$Cr labeling was achieved by incubating $5 \times 10^7$ cells/ml for 45 min at 37°C in RPMI-FCS containing 40 μCi/ml. The cells were washed four times in RPMI-FCS and resuspended for intravenous injection. $[^{3}H]$-labeling was achieved by incubating $5 \times 10^7$ spleen cells/ml for 1 h at 37°C in RPMI-FCS containing 5 μCi of $[^{3}H]$thymidine per ml. The cells were washed four times with RPMI-FCS. Each recipient received $10^7$ $^{51}$Cr-labeled cells or $5 \times 10^7$ $[^{3}H]$-labeled cells intravenously in a volume of 0.2 ml. $^{51}$Cr in blood, spleen, liver, lungs, and kidneys was counted with a gamma counter (model 1185, Searle, Des Plaines, IL). $[^{3}H]$-associated acid-precipitable material from known weights of spleen and liver were counted in a scintillation spectrometer (model LS7500, Beckman Instruments, Wakefield, MA) according to a published method (6).

**Statistics.** Linear regression analysis of data was performed using a programmable calculator (EC 4000 programmable calculator, Radioshack, Tandy Corporation, Fort Worth, TX).

**Results**

In a previous publication (4), it was shown that spleen cells from *Listeria*-immune senescent mice show a profoundly decreased capacity to adoptively immunize normal syngeneic recipients than cells from young immune donors. It was possible that cells from senescent immune donors failed to transfer normal levels of protection because of the presence of immunoregulatory cells that inhibit the full expression of protective activity of anti-Listeria mediator T cells. To test this possibility, a fixed number of spleen cells from day-6 *Listeria*-immune young mice (indicator cells) was mixed with different number of spleen cells from either normal or day-6 *Listeria*-immune senescent mice. The protective capacity of resultant cell mixture was compared in an adoptive immunization assay with equivalent number of spleen cells from young or senescent immune donors. As can be seen in Table I, addition of 6–25 million spleen cells from normal or immune senescent mice did not significantly ($P > 0.05$) reduce the level of protection conferred with 25 million spleen cells from young immune donors. The same result was obtained in other experiments in which the number of indicator cells was either reduced to 12 million or increased to 50 or 100 million. Also, T-enriched or unfractionated whole spleen cells gave identical results (results not shown).

**Dose-Response Analysis of Protective T Cells from Young and Senescent Donors.** Two possibilities were considered to seek an explanation for the decreased capacity of spleen cells from senescent immune donors to adoptively immunize young recipients: (a) it was possible that the senescent donors generated fewer protective T cells in response to immunizing infection, and (b) the protective T cells from senescent donors
Evidence That Cells from Normal or Immune Senescent Mice Do Not Show a Suppressive Effect on the Protective Capacity of Cells from Young Immune Mice

Table I

| Number of Listeria-immune spleen cells (x 10^6) | Log_10 protection |
|-----------------------------------------------|-------------------|
| Young donors | Old donors |
| 12.5 | 1.24 |
| 25.0 | 2.60 |
| 50.0 | 3.40 |
| -- | 0.18 |
| -- | 0.40 |
| -- | 0.52 |
| 25.0 mixed with 6.0* | 2.70 |
| 25.0 mixed with 12.5* | 2.82 |
| 25.0 mixed with 25.0* | 2.75 |
| 25.0 mixed with 6.0 | 2.97 |
| 25.0 mixed with 12.5 | 2.82 |
| 25.0 mixed with 25.0 | 2.80 |

* Cells from normal senescent donors.

on a per cell basis might be less efficient at transferring protection than cells from young immune donors. A dose-response analysis revealed that there was a log linear relationship between the number of spleen cells transferred and log protection conferred upon recipients with cells from young donors but not with cells from senescent donors (Fig. 1). The regression coefficient value obtained with data pooled from eight separate experiments was 0.85 for cells from young donors and 0.30 for cells from senescent donors. Moreover, it required at least 75 million cells from senescent donors to transfer a minimum significant level of protection. Compared with this, as few as 150 million cells from young donors transferred a level of protection that reached the maximum limit of detection.

A Comparison between Homing Pattern of Protective T Cells from Young and Senescent Donors.

Another possible reason that cells from senescent immune donors transferred a lower level of protection than cells from young immune donors was that they distributed differently into recipient mice. To investigate this possibility, cell transfer experiments were performed with enriched ^51^Cr-labeled splenic T cells from young or senescent donors. At progressive times after intravenous infusion, recipients were killed and the level of radioactivity measured in various organs. Radioactivity disappeared from the blood in 30 min, most of which rapidly became concentrated in the lungs. A progressive decrease in radioactivity in the lungs over the first 6 h was accompanied by a progressive increase in radioactivity in the liver and spleen (Fig. 2, left panel). After 6 h there was a slow loss of radioactivity from all organs. No significant differences were detected in the levels of radioactivity between the organs of recipient mice infused with splenic T cells from either young or senescent donors. In spite of identical homing patterns, the same cell populations exhibited profound differences in their protective capacities (Fig. 2, right panel).

^51^Cr label was chosen because it makes it relatively simple to monitor various organs for radioactivity distribution. It could be argued, however, that ^51^Cr does not monitor the effector T cells because they are so diluted out with ^51^Cr-labeled nonspecific T cells. It is well established (6, 7) that mediator T cells of anti-Listeria immunity are actively dividing immunoblasts. Accordingly, in the next experiment ^51^Cr was replaced by [^3H]thymidine as a label to confirm the results with ^51^Cr-labeled...
cells by those obtained with $^{3}H$-labeled cells (results not shown).

**Passive Transfer of Anti-Listeria Immunity between Young and Senescent Mice.** All the evidence for impaired ability of cells from senescent immune donors to transfer protection was derived from the cell transfer experiments that used only young mice as recipients. It was important to determine whether the same result could be obtained if the cells were transferred to senescent recipients. Accordingly, reciprocal cell transfers were performed by infusing young or senescent mice with cells from day-6 immune young or senescent donor mice. As can be seen in Table II, cells from senescent immune donors showed markedly lowered capacity to transfer protection than cells from young immune donors, regardless of the age of recipient mice.

**Discussion**

These studies confirm results presented in a previous report that showed that protective T cells from young mice were at least 1,000 times more capable of...
Passive Transfer of Anti-Listeria Immunity between Young and Senescent Mice

| Source of Listeria-immune spleen cells* | 48-h log_{10} Listeria counts/spleen† | Young recipients | Old recipients |
|----------------------------------------|---------------------------------------|-----------------|---------------|
| None                                   | 6.31 ± 0.08                           | 6.31 ± 0.10     |
| Young mice                             | 3.51 ± 0.14                           | 3.42 ± 0.59     |
| Old mice                               | 5.25 ± 0.07                           | 5.22 ± 0.38     |

* 10^8 spleen cells from day-6 Listeria-immune donors were infused intravenously into recipients that were challenged with 5 x 10^4 Listeria 1 h later.
† Results expressed as mean ± SD.

Transferring protection than equivalent number of spleen cells from senescent donors. The reduced protective capacity of spleen cells from senescent donors could not be attributed to (a) inhibition of their activity by suppressor cells nor (b) differences in the “homing patterns” of these cells into recipients. Additional support for a reduced capacity of protective T cells from senescent mice came from reciprocal cell transfer experiments. It was found that protective cells from young donors transferred substantially more protection than cells from senescent donors, even when the cells were infused into senescent recipients. Results of these experiments revealed another important point: given the appropriate conditions, senescent mice do possess the normal capacity to mobilize and activate mononuclear phagocytic cells.

The differences in the protective capacity of immune spleen cells from the two age groups could not be explained solely on the basis of differences in the absolute number of protective T cells. This was revealed by a dose-response analysis, an approach that was used previously (8) to compare the efficiency of protective T cells from different compartments of Listeria-immune host. Using this approach, it was found that on a per cell basis, protective cells from young donors out-performed protective cells from aged donors by at least a factor of 100. For example, to transfer a minimum statistically significant level of protection, it required 80–100 million spleen cells from aged mice compared with only 8–10 million cells from young donors. Assuming that the per cell basis efficiency for both the cell populations was the same, this would indicate that the spleens from young donors contained about 10 times more protective T cells than aged mice. However, 100 million immune cells from the young donors transferred about 1,000-fold more protection than equivalent number of spleen cells from aged mice. This implies that cells from young donors were at least 100 times more efficient in transferring protection. This conclusion was reinforced by the results obtained with T-enriched immune spleen cells, providing convincing evidence that the lower per cell protective capacity of spleen cells from senescent mice could not be attributed to the dilution effect rendered by the larger than normal proportion of irrelevant non-T cells.

Though immunity to listeriosis is mediated by a population of actively dividing T cells, it is expressed by activated macrophages (1, 7, 9). The activation of macrophages is undoubtedly achieved through the production of soluble mediators (lymphokines) that are released upon interactions between the antigen and sensitized T cells (9, 10). Thus, the lowered efficiency of protective T cells from senescent mice could result from quantitative and/or qualitative differences in the lymphokine production. Recently published (11, 12) evidence lends some support to this hypothesis.
Summary

Experimental murine listeriosis was used as a model to investigate the immunological basis for the age-associated decline in antimicrobial immunity. The reduced capacity of protective T cells from Listeria-immune senescent mice to adoptively immunize normal syngeneic recipients could not be attributed to inhibition of their activity by suppressor cells. Radiolabeled enriched splenic T cells from Listeria-immune young or senescent donors exhibited an identical distribution pattern after an intravenous infusion into young recipients. Moreover, cells from Listeria-immune young donors showed markedly greater protective capacity than cells from senescent immune donors whether the cells were transferred to young or senescent recipients. Dose-response analysis of protective T cells revealed that in response to immunizing infection (a) senescent mice generated 10-fold fewer protective T cells, and (b) protective T cells from senescent mice were 100-fold less efficient than cells from young mice.

The expert technical assistance of Susan M. Knobel is gratefully acknowledged.

Received for publication 7 December 1981 and in revised form 29 March 1982.

References

1. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. 129:937.
2. North, R. J. 1974. Cellular immunity and the response to infection. In Mechanisms of Cellular Immunity. R. T. McCluskey and S. Cohen, editors. John Wiley and Sons, Inc., New York. 185–218.
3. Patel, P. J. 1981. Aging and cellular defense mechanisms: age-related changes in resistance of mice to Listeria monocytogenes. Infect. Immun. 32:557.
4. Patel, P. J. 1981. Aging and antimicrobial immunity: impaired production of mediator T cells as a basis for the decreased resistance of senescent mice to listeriosis. J. Exp. Med. 154:821.
5. Smith, G. S., R. L. Wallford, and M. R. Mickey. 1973. Life span and incidence of cancer and other diseases in selected long lived inbred mice and their F1 hybrids. J. Natl. Cancer Inst. 50:1195.
6. North, R. J. 1971. The action of cortisone acetate on cell-mediated immunity to infection. Suppression of host cell proliferation and alteration of cellular composition of infective foci. J. Exp. Med. 134:1485.
7. North, R. J. 1973. Cellular mediators of anti-Listeria immunity as an enlarged population of short-lived replicating T-cells. Kinetics of their production. J. Exp. Med. 138:342.
8. North, R. J., and G. L. Spitalny. 1974. Inflammatory lymphocyte in cell-mediated antibacterial immunity: factors governing the accumulation of mediator T-cells in peritoneal exudates. Infect. Immun. 10:489.
9. Mackaness, G. B. 1973. Technical report series No. 59. Cell-mediated immunity and resistance to infection. World Health Organization, Geneva.
10. Farr, A. G., M. E. Dorf, and E. R. Unanue. 1977. Secretion of mediators following T-lymphocyte-macrophage interaction is regulated by the major histocompatibility complex. Proc. Natl. Acad. Sci. U. S. A. 74:1977.
11. Miller, R. A., and O. Stutman. 1981. Decline, in aging mice, of the anti-2,4,6-trinitrophenyl (TNP) cytotoxic T-cell response attributable to loss of Lyt-2", interleukin-2 producing helper cell function. Eur. J. Immunol. 11:751.
12. Thoman, M. L., and W. O. Weigle. 1981. Lymphokines and aging: interleukin-2 production and activity in aged animals. J. Immunol. 127:2102.