CELLULAR IMMUNOLOGY **118**, 229–233 (1989)

SHORT COMMUNICATION

Aging and Immunity to Tuberculosis: Prolonged Survival of Old Mice Infected with *Mycobacterium tuberculosis* by Adoptive Immunization with Memory-Immune T Lymphocytes

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Received June 30, 1988; accepted September 16, 1988

This study shows that memory immune T lymphocytes, harvested from young (3-month-old) mice infected intravenously with *Mycobacterium tuberculosis* and then exposed to protracted chemotherapy with isoniazid, are capable of adoptively protecting old (24 month) mice from a subsequent fatal challenge infection. Survival of such adoptively protected animals was prolonged, but did not extend beyond the mean survival time of uninfected old control mice. During this time the passively transferred memory T cell population retained their functional capacity to protect against subsequent tuberculosis infection. These data indicate, therefore, that reconstitution of decayed cell-mediated antimicrobial immunity in old mice *in vivo* with memory T cells is technically feasible, although the life span of the animal is not extended over that of control animals. They indicate, moreover, that the memory T cells remain functional in what some reports have considered a suppressive environment and show further that the macrophages with which the infused T cells interact in the aged host remain functionally able to express acquired resistance.

INTRODUCTION

It is a general belief that susceptibility to tuberculosis increases with age. In animal models this belief is borne out by evidence that shows that the capacity of mice to generate protective T lymphocytes against *Mycobacterium tuberculosis* infection exhibits an age-related decline, with animals of approximately 2 years of age no longer able to achieve this function (1). Moreover, at about this time, such mice begin to show evidence of recrudescence of pulmonary disease, in situations where the tuberculosis infection had been introduced while the animal was young (2).

In the present study, attempts were made to protect old mice from the fatal effects of *M. tuberculosis* infection by infusing them with memory immune T cells from young mice that had been infected with tuberculosis, thus exploiting the long-lived characteristic of such cells (3). The results of these experiments show that such adoptive protection was successful in prolonging the survival of such animals to mean life spans comparable to those shown by uninfected control animals.

MATERIALS AND METHODS

Mice. These experiments were performed using B6D2 (C57BL/6 × DBA/2)F_1 female specific-pathogen-free hybrid mice. They were purchased as young mice from

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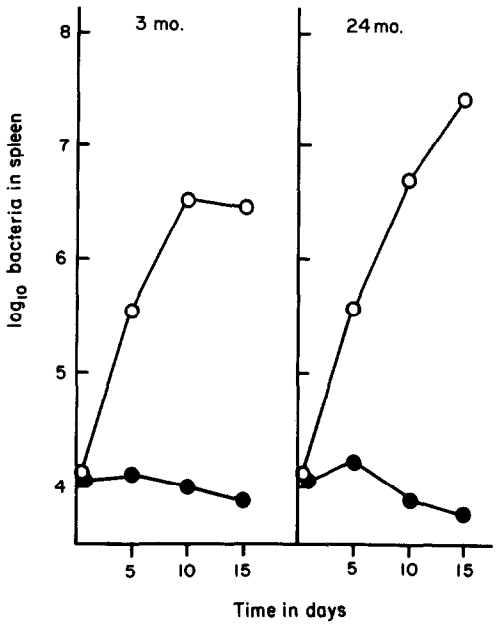

FIG. 1. Demonstration of the capacity of memory-immune T cells to adoptively protect both young and old mice against *M. tuberculosis* challenge. Data shown as mean values ($n = 4$; SEM omitted; they did not exceed 0.30). Recipients were given normal T cells (○) or memory-immune (●) T cells.

the Trudeau Institute (Saranac Lake, NY). Aging mice were handled and treated with extreme care, as previously described (2).

Bacterial infections. Mice were infected intravenously via a lateral tail vein with indicated numbers of *M. tuberculosis* strain Erdman. The organism was grown to mid-log phase in Proskauer Beck medium and then stored in 5-ml aliquots at −70°C until required. For memory-immune donors, 3-month-old donor mice were infected intravenously with 1×10^5 viable *M. tuberculosis* and then 25 days later exposed to isoniazid chemotherapy (100 mg/liter drinking water). Ninety days later the therapy was discontinued, and mice were used as memory T cell donors a further 10 days later (4). The course of tuberculosis infections was followed against time by plating serial dilutions of individual whole organ homogenates on nutrient Middlebrook 7H11 agar and counting bacterial colony formation after 14–20 days incubation at 37°C in humidified air.

Passive cell transfer experiments. Spleen cell suspensions were prepared from memory cell donors and depleted of adherent cells by incubation on plastic petri dishes for 2 hr at 37°C in 7% CO_2. The medium used was RPMI 1640 culture medium containing 1 m*M* glutamine and 2.5% fetal calf serum. Nonadherent cells were washed and then treated with monoclonal J11d.2 antibody (5) plus complement in a one-step procedure (1) to enrich for T cells. Recipient mice were infused intravenously with one spleen equivalents of T cell-enriched spleen cells, followed by challenge with 1×10^5 *M. tuberculosis* at the times indicated. Mice were exposed to 500 rad whole-body ionizing γ irradiation by a ^{137}Ce source at a dose rate of 80 rad/min prior to use as recipients in passive transfer assays. Only young mice required irradiation to be used as recipients of cells; we have recently discovered that the radio-

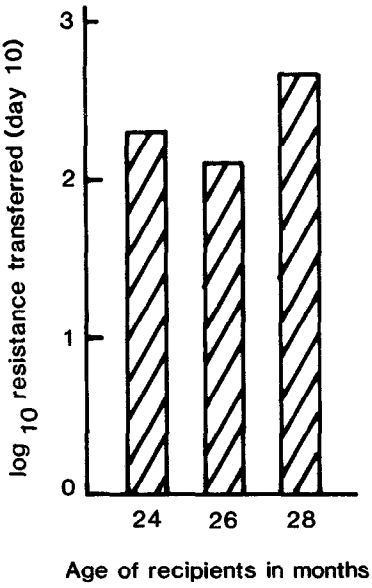

FIG. 2. Evidence that memory-immune cells can remain functional for at least 4 months following infusion into 24-month-old mice. Resistance values calculated on Day 10 of challenge infection ($n = 4$).

sensitive isogeneic barrier in mice to adoptive immunization is lost in the old (24 month) mice, thus removing the need for their sublethal irradiation prior to cell infusion (I. M. Orme, submitted for publication).

RESULTS

Protection of old mice by memory T cells. In an initial series of experiments, the ability of memory T cells to adoptively protect young and old recipients of these cells was compared. It was found (Fig. 1) that in both groups of animals the memory cells conferred the ability to exert bacteriostasis against the challenge infection. It will be noted, in addition, that this challenge inoculum grew progressively in the old recipient mice, while the growth of the organism slowed in young mice after 14 days of the infection. Experiments were then performed to determine if transferred cells were able to remain functional over a given period of time in the absence of antigenic stimulation in the old recipient animals. To achieve this, uninfected old mice were infused with memory T cells and left for a period of time prior to challenge. During this time these mice were given isoniazid therapy (until 7 days prior to challenge) to exclude the unlikely possibility that small numbers of contaminating bacteria in the cell inoculum which had escaped the effects of chemotherapy in the donor animals might actively immunize the recipients. The results of these experiments (Fig. 2) show that the memory-immune T cells retained their capacity to respond to the delayed infectious challenge for the 4 months of the experimental period.

Survival of adoptively protected old mice. In a third series of experiments, it was determined to what extent old infected mice would be allowed to survive by means of the adoptive protection procedure. In these experiments it was found that while 24-month-old infected, unprotected mice died rapidly from the tuberculosis infection,

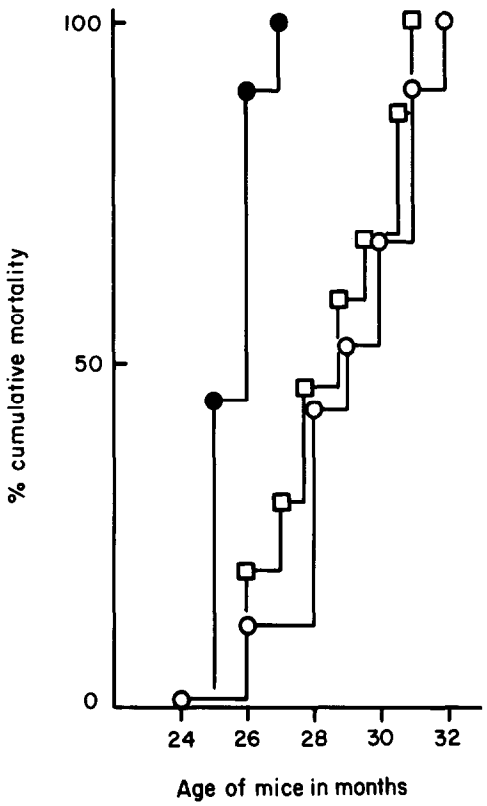

FIG. 3. Cumulative mortality of old mice following infection with *M. tuberculosis* alone (●), infection 2 days following infusion of mice with memory-immune cells (□), or controls (○). $n = 20$ for each group.

infected mice infused with memory T cells lived on average 4 to 5 months longer, to the extent that their mean survival time was comparable to that of uninfected age-matched control mice (Fig. 3).

DISCUSSION

The results of this study therefore show that memory T cells, obtained from young mice, are capable of adoptively protecting old mice from the lethal effects of a *M. tuberculosis* challenge. Not only are the transferred cells fully functional in the old mice, but they retain this capacity in these mice if the challenge infection is delayed by as much as 4 months. Such animals do not survive longer than uninfected controls, however, indicating that other intrinsic physiological events subsequently presumably lead to the demise of the animal.

These results, therefore, further confirm the finding that the age-related decline in antituberculous immunity in these animals can be artificially reversed by the passive transfer of immune cells from young mice, as previously observed in this laboratory (1). They further extend these observations, however, by showing that such transferred immunity can functionally persist in the recipient animals. This finding is of importance, in view of the results of previous studies that have indicated that de-

creased cell-mediated immunity in old mice is temporally associated with a concomitant increase in suppressor T cell activity (6–10). The present result excludes the possibility, therefore, that should such suppressor T cells persist in 24-month-old animals, their range of activity does not extend to this particular anti-microbial memory T cell population. These data, moreover, indicate that macrophages in the aging host also remain capable of expressing acquired resistance, when prompted to do so by functional T cells.

Currently, the precise reasons for the decline in antituberculous immunity remains unclear. It may reflect intrinsic deficiencies (11), such as a decline in the frequency of precursor cells that give rise to the mediators of antituberculous immunity; in other systems such declines have been documented, although not always in conjunction with a loss of responsiveness (6). On the other hand, cells capable of giving rise to such immunity may not actually be lost in the old animals, but may instead lack, or have become unresponsive to, relevant triggering signals. This particular hypothesis is partially supported by evidence that the T cells of old mice are in fact highly responsive to infection with another intracellular parasite, *Listeria monocytogenes* (12). Such findings, taken together, suggest that in addition to reconstitution of the animal with immune cells, an alternative strategy for the protection of such mice against tuberculosis might consist of exploring procedures whereby the activity of such putative unresponsive cells might be restored.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grant AG-06946. I thank Alan Roberts for excellent technical assistance.

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