

DEFICIENCY IN SUPPRESSOR T CELL ACTIVITY IN AGED
ANIMALS

Reconstitution of this Activity by Interleukin 2*

By MARILYN L. THOMAN^{†§} and WILLIAM O. WEIGLE

From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Advancing age is accompanied by declining immune potential. Numerous investigations have pinpointed deficiencies in the activity of B lymphocytes and several T lymphocyte subsets (reviewed in references 1 and 2) in aged animals and humans. In contrast, it has been suggested that increased T suppressor cell activity might be responsible for the depressed immunity of aged animals, and in fact, increased suppressor cell activity has been reported (3-8), although decreased suppressor activity has also been documented (9, 10). Our previous work (11-13) has demonstrated that aged T cells are unable to produce adequate amounts of the T cell growth-promoting lymphokine, interleukin 2 (IL-2), and it is this deficiency that is largely responsible for the lowered immune potential of aged animals. Supplementation of spleen cells from aged animals with IL-2 stimulates the normal, high level of responsiveness in such systems as the mixed lymphocyte reaction and the generation of cytotoxic T cells, and largely restores the anti-sheep erythrocyte (SRBC) plaque-forming cell (PFC) response. These data clearly demonstrate that aged animals possess adequate numbers of precursor T cells of the helper and cytotoxic sets, and that in the presence of IL-2 and antigen these cells expand and are functional. In this report we document that aged spleen cells are deficient in suppressor cell activity, but this lesion can be corrected by supplementation with IL-2, using a suppressor-generation system reported by Green et al. (14). This system has the significant advantage that the suppressors are generated during a first culture period, then mixed with young adult cells to test their activity, thus avoiding possible interactions between young and old cells during the induction of the suppressor cells.

Materials and Methods

Mice.

Male C57Bl/6Cr mice at 3-6 mo of age were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, MA.

Generation and Assay of Suppressor Cell Activity.

The protocol utilized was essentially that described by Green et al. (14) in which spleen cells are cultured for a period of 3-4 d to generate suppressor cells. These suppressors are co-cultured for a brief period (48 h) with T cells of the helper (inducer) phenotype, and then the function of these cells is assayed by co-culture with B cells and antigen.

* Supported in part by grants AI07007, AG00783, AI15761, and Biomedical Research Support Grant RRO-5514 from the U. S. Public Health Service.

† Recipient of U. S. Public Health Service National Research Service Award AI06085.

§ To whom correspondence should be addressed.

SUPPRESSOR GENERATING CULTURE. Unfractionated murine spleen cells were cultured at a density of 5×10^6 /ml 3–5 d in complete media consisting of RPMI 1640 supplemented with 7.5% fetal calf serum, 200 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 5×10^{-6} M 2-mercaptoethanol (2-ME). To some cultures 10 U of IL-2 was added at culture initiation. At the conclusion of this period the cells were collected, washed, and treated with anti-Ly-1.2 and complement (C'), then used in the intermediate culture phase.

GENERATION OF PRIMED LY-1⁺ T CELLS. 12–16-wk old C57Bl/6 mice were immunized with one intraperitoneal injection of 0.2 ml of 0.1% SRBC. 4 d later the spleens were removed and the T cells collected by nylon-wool column purification (15). These T cells were then treated with anti-Ly-2 and C' to deplete them of suppressor cells and the remaining cells were mixed with cells from the suppressor-generation cultures.

INTERMEDIATE CULTURES. 2.5×10^6 Ly-1⁺2⁻ T helper cells were cultured with various numbers of the anti-Ly-1 and C'-depleted T suppressor cells, as given in Table I, for a period of 48 h. The cells were then collected and treated with anti-Ly-2.2 and C' to remove suppressor cells. The control cells, Ly-1⁺2⁻ helper T cells cultured in the absence of suppressor cells, were also treated with the antisera and C' to control for nonspecific effects of this treatment. The remaining cells were assessed for helper activity.

ASSAY CULTURE. B cells were prepared by treatment of 12–16-wk old C57Bl/6 spleen cells with anti-Thy-1.2 and C'. Viable cells were recovered from the interface of Ficoll-Isopaque density gradients. 5×10^6 B cells were cultured with 5×10^6 SRBC and $1-2.5 \times 10^5$ T cells from the intermediate cultures. Controls included (a) culturing the B cells alone to assess the efficacy of the anti-Thy treatment, and (b) to confirm that no suppressor activity remained after treatment of the cells in the intermediate cultures with anti-Ly-2 and C', co-culturing these cells with B cells and Ly-1⁺ helpers incubated in the absence of Ts during the intermediate cultures and B cells. In no instance did this control indicate residual suppressor activity remaining in the anti-Ly-2-treated populations. Anti-SRBC PFC were enumerated on day 5 by the slide modification of the Jerne plaque assay.

Preparation and Quantitation of IL-2.

Spleen cells were cultured at a density of 1×10^6 cells/ml in RPMI 1640 supplemented with 0.01 M Hepes, 200 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, 5×10^{-6} M 2-ME, and 2 μ g/ml concanavalin A. Following a 24-h culture period, cell-free supernatant was collected, concentrated by vacuum dialysis, and applied to a 2.5 cm \times 90-cm column of (LKB Instruments, Inc., Rockville, MD) AcA54. The column was eluted with sterile saline and 7-ml fractions were collected. Each fraction was tested for IL-2 activity in a T cell growth assay using CTLL-2 cells. Active fractions were pooled sterile filtered (0.22 μ Nalgene Type S Filter unit, Nalgene Labs., Rochester, NY) and frozen, until used. IL-2 activity was quantitated as described by Farrar et al. (16), using a standard preparation containing an arbitrarily assigned value of 100 U/ml.

Results

Generation of Suppressor Cell Activity from Young and Aged Animals. Spleen cells from young animals, when cultured 3–5 d, give rise to cells that are able to suppress the activity of antigen-specific helper T cells, as has been described by several groups (13, 17–19). When aged spleen cells are cultured in a similar fashion, far less suppressor activity is generated compared with cells from young adults (Table I). Similar decreases in the activity of T cells of other functional subsets have been described (20–23), and our recent results suggest that a common lesion is the inability of aged T lymphocytes to produce IL-2. Upon addition of IL-2 to cultures containing aged cells, T cell-dependent immune responses are markedly enhanced (11, 12). To determine whether the deficiency in generation of suppressors from aged animals is also affected by IL-2, suppressor-generating cultures were set up containing IL-2. 10 U of IL-2 were added to cultures containing 5×10^6 spleen cells. Following the 3–4-d generation phase, the cells were collected, and tested as described in Materials and

TABLE I
Generation of Suppressor Cell Activity from Spleen Cells of Aged and Young Adult Animals

Cells in intermediate culture*		IL-2 [‡]	PFC/10 ⁶ ± SE		
Ly 2 ⁺ Ts	Ly 1 ⁺ TH		Experiment 1	Experiment 2	Experiment 3
—	+	—	1,300 ± 130	920 ± 24	2,400 ± 161
Aged	+	—	350 ± 70	824 ± 48	1,105 ± 45
Aged	+	+	80 ± 60	40 ± 10	367 ± 45
Young	+	—	70 ± 30	21 ± 3	87 ± 13
Young	+	+	20 ± 15	4 ± 2	87 ± 13

* 2.5 × 10⁶ T helper cells were cultured with 1-20 × 10⁵ Ly-2⁺ T cells from the suppressor-generating cultures. After 48 h of co-culture, cells were harvested and treated with α-Ly-2 and C'. Helper activity was assayed as described in the Materials and Methods.

[‡] 10 U were added at the initiation of the suppressor-generation culture period.

Methods. As shown in Table I, although aged spleen cells are normally deficient in the generation of Ts, upon addition of IL-2 such cells are readily formed. For example, Lyt-2⁺ Ts cells generated from aged animals in the absence of IL-2 decrease the helper function of Lyt-1⁺ cells by 54%, Lyt-2⁺ Ts from aged animals generated in the presence of IL-2 inhibit helper function of Lyt-1⁺ cells 85%, and Ts from young animals (generated either in the presence or absence of IL-2) suppress 96% of the helper activity. Thus, while IL-2 has little or no effect on the number of suppressor cells generated by culturing spleen cells from young adult animals, it markedly increased the number generated from aged spleen cells. Thus, IL-2 reconstitutes the ability to produce Ts.

Quantitation of Suppressor Cell Activity. To quantitate the amount of suppression generated in the presence of IL-2 by aged and young adult animals, the Ts-containing cells were co-cultured with the Lyt-1⁺ helper cells in decreasing concentrations. Thus, a constant number (2 × 10⁶) Lyt-1⁺ cells were cultured with either 1 × 10⁵, 5 × 10⁴, or 1 × 10⁴ Lyt-2⁺ Ts cells in the intermediate culture phase. The degree of help exerted by the Lyt-1⁺ cells after the intermediate culture was then determined. As shown in Fig. 1, when Lyt-1⁺ helpers are co-cultured with 1 × 10⁵ or 5 × 10⁴ Ts, the aged suppressors generated in the presence of IL-2 are just as effective as cells derived from young adult animals. At 1 × 10⁴, the aged Ts are somewhat less effective than the young Ts. However, compared with the aged suppressors generated in the absence of IL-2, this number of aged Ly-2⁺ cells cultured in the presence of IL-2 are three times more inhibitory. Thus, IL-2-treated aged spleen cells effect 85% suppression and IL-2-treated young spleen cells effect 96% suppression.

Discussion

These data indicate that spleens of aged animals are less capable of giving rise to T suppressor cells when cultured in vitro. Further, this deficiency can, to a large degree, be corrected by adding partially purified lymphokine preparations containing IL-2 to such cultures. Thus, the generation of Ts is similar to other T cell-dependent immune functions of aged spleen cells, such as the generation of cytotoxic T lymphocytes, the mixed lymphocyte reaction, and the anti-SRBC response, which are all enhanced by addition of IL-2-containing lymphokine preparations (11, 12, 24).

The active moiety in the lymphokine preparations has not been definitely identified as IL-2, although this factor is a major component. Thus, it cannot be ruled out that

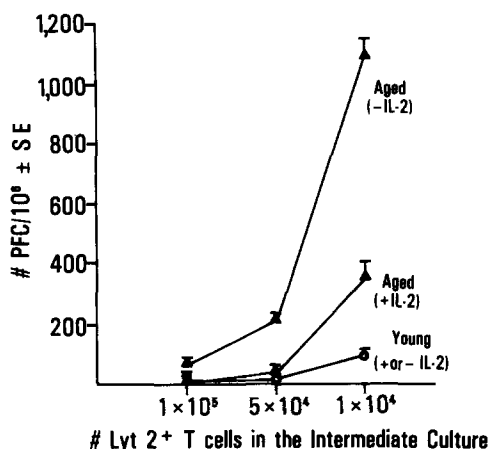


FIG. 1. Titration of suppressor activity from aged and young adult spleen cells cultured in the presence or absence of IL-2. Suppressor cells were generated as described in Materials and Methods. These cells were treated with anti-Lyt-1 and C' after a 4-d culture period and admixed with Lyt-1⁺T helper cells. 2.5×10^6 helper cells were co-cultured with 1×10^5 , 5×10^4 , or 1×10^4 Lyt-2⁺ suppressor cells. The helper activity remaining after a 48-h intermediate culture was assessed. The positive control consisting of 5×10^6 B cells and 5×10^5 Lyt⁺ T helper cells cultured in the absence of suppressor cells gave $2,400 \times 10^6$ PFC/ 10^6 B cells.

other factors may be important in the reconstitution of these immune responses by aged animals. However, it is well documented that T lymphocytes of the helper, cytotoxic, and suppressor functional subsets apparently bear receptors for IL-2, as they may be grown for long periods in the presence of this lymphokine (25-28). Furthermore, these experiments do not identify the target of the lymphokine(s) within the spleen cell population. That is, the factor may act directly upon the precursor of the suppressor cell, or another cell necessary for the generation of the Ts.

Our results differ from several other reports which conclude that T cell activity is unimpaired or increased in aged animals (3-7). Significant differences exist in the experimental protocols used by these groups and the suppressor system used in our experiments. Perhaps the most important element is that in other systems, aged and young cells are co-cultured for the entire assay period, possibly allowing lymphokines generated by the young cells to exert important effects on the aged cells. It is clear that in the experimental set-up used in this report, suppressor cells from aged cells are deficient in their expansion during a 3-5-d period in vitro, and that lymphokine preparations containing IL-2 restore this function. In this respect our results are reminiscent of others (8, 9) that demonstrate that splenocytes from aged animals are not as effectively stimulated by concanavalin A to become suppressor cells as are cells from young adult animals. This may also reflect the relative inability of aged cells to produce IL-2 and/or other lymphokines upon stimulation with mitogens. These data suggest that aged animals possess cells belonging to the suppressor circuit that cannot be optionally expressed as a result of the inability of aged animals to produce IL-2.

Summary

Spleen cells derived from aged (30 mo) C57Bl/6 mice are shown to be deficient in the ability to generate suppressor cell activity in vitro. the addition of IL-2 to cultures containing aged cells restores this function to a large extent.

Received for publication 4 February 1983.

References

1. Makinodan, T., and M. Kay. 1980. Age influence on the immune system. *Adv. Immunol.* **29**:287.
2. Kay, M., and T. Makinodan. 1981. Relationship between aging and the immune system. *Prog. Allergy.* **29**:134.
3. Goidl, E. A., J. B. Innes, and M. E. Weksler. 1976. Immunological studies of aging. II. Loss of IgG and high avidity plaque-forming cells and increased suppressor cell activity in aging mice. *J. Exp. Med.* **144**:1037.
4. Segre, D., and M. Segre. 1976. Humoral immunity in aged mice. II. Increased suppressor T cell activity in immunologically deficient old mice. *J. Immunol.* **116**:735.
5. Roder, J., D. Bell, and S. Singhal. 1977. Regulation of the immune response in autoimmune NZB/NZW F1 mice. I. The spontaneous generation of splenic suppressor cells. *Cell. Immunol.* **29**:272.
6. DeKruyff, R., Y. Kim, G. Siskind, and M. Weksler. 1980. Age-related changes in the *in vitro* immune response: increased suppressor activity in immature and aged mice. *J. Immunol.* **125**:142.
7. Lui, J., M. Segre, and D. Segre. 1982. Changes in suppressor, helper, and B cell functions in aging mice. *Cell. Immunol.* **66**:372.
8. Doria, G., C. Mancini, and L. Adorini. 1982. Immunoregulation in senescence: increased inducibility of antigen-specific suppressor T cells and loss of cell sensitivity to immunosuppression in aging mice. *Proc. Natl. Acad. Sci. USA.* **79**:3803.
9. Krakauer, R. S., T. A. Waldmann, and W. Strober. 1976. Loss of suppressor T cells in adult NZB/NZW mice. *J. Exp. Med.* **144**:662.
10. Hallgren, H., and E. Yunis. 1977. Suppressor lymphocytes in young and aged humans. *J. Immunol.* **118**:2004.
11. Thoman, M., and W. Weigle. 1981. Lymphokines and aging: interleukin 2 production and activity in aged animals. *J. Immunol.* **127**:2102.
12. Thoman, M., and W. Weigle. 1982. Cell-mediated immunity in aged mice: an underlying lesion in IL-2 synthesis. *J. Immunol.* **128**:2358.
13. Morgan, E., and W. Weigle. 1982. The immune response in aged C57Bl/6J mice. II. Characterization and reversal of a defect in the ability of aged spleen cells to respond to the adjuvant properties of Fc fragments. *J. Immunol.* **129**:36.
14. Green, D., R. Gershon, and D. Eardley. 1981. Function deletion of different Ly-1 T cell-inducer subset activities by Ly-2 suppressor T lymphocytes. *Proc. Natl. Acad. Sci. USA.* **78**:3819.
15. Julius, M., E. Simpson, and L. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
16. Farrar, J., S. Mizel, J. Fuller-Farrar, W. Farrar, and M. Hilfiker. 1980. Macrophage-independent activation of helper T cells. I. Production of interleukin 2. *J. Immunol.* **125**:793.
17. Hodes, R., and K. Hathcock. 1976. *In vitro* generation of suppressor cell activity: suppression of *in vitro* induction of cell-mediated cytotoxicity. *J. Immunol.* **116**:167.
18. Ferguson, R., S. Anderson, and R. Simmons. 1978. Characterization of suppressor lymphocytes generated *in vitro*. *Transplantation (Baltimore).* **26**:331.
19. Anaderio, A., M. Moras, C. Honorati, A. Ruggeri, G. Conti, and F. Spreafico. 1979. *In vitro* generation of nonspecific suppressor cells and their characterization. *Transplantation (Baltimore).* **27**:329.
20. Heidrick, M., and T. Makinodan. 1973. Presence of impairment of humoral immunity in nonadherent spleen cells in old mice. *J. Immunol.* **111**:1502.
21. Konen, T., G. Smith, and R. Walford. 1973. Decline in mixed lymphocyte reactivity of spleen cells from aged mice of a long-lived strain. *J. Immunol.* **110**:1216.
22. Bach, M. 1977. Lymphocyte-mediated cytotoxicity: effects of aging adult thymectomy and thymic factor. *J. Immunol.* **119**:641.

23. Krosgrud, R., and E. Perkins. 1977. Age-related changes in T cell function. *J. Immunol.* **118**:1607.
24. Miller, R., 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.
25. Gillis, S., and K. Smith. 1977. Long-term culture of tumor-specific cytotoxic T cells. *Nature (Lond.)*. **268**:154.
26. Watson, J. 1979. Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. *J. Exp. Med.* **150**:1510.
27. Gillis, S., P. E. Baker, F. W. Ruscetti, and K. A. Smith. 1978. Long-term culture of human antigen-specific cytotoxic T-cell lines. *J. Exp. Med.* **148**:1093.
28. Levich, J., W. Weigle, and D. E. Parks. 1982. Functional and phenotypic analysis of long-term suppressor cell lines cultured with antigen. *Immunobiology*. **163**:268.