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EFFECTS OF LONG-TERM TREATMENT OF MICE WITH ANTI-I–J MONOCLONAL ANTIBODY AND DIALYZABLE LEUKOCYTE EXTRACT ON IMMUNE FUNCTION AND LIFESPAN

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SUMMARY

In 1969 Walford hypothesized that age-related dysfunctions of the immune system may be involved in the pathogenesis of the lesions and diseases of aging. Studies were initiated to test whether immunologic interventions intended to maintain the integrity of the immune system would delay the onset of diseases of aging and prolong lifespan. Adult BC3F1 mice were treated with anti-I–J monoclonal antibody, with human dialyzable leukocyte extract, or with saline once a week for one year. Spleen cells from the mice were then assayed for suppressor, T-helper and B-cell activity. Treatment with dialyzable leukocyte extract decreased the elevated nonspecific suppressor activity. Mice treated with anti-I-J antibody had elevated T-helper cell activity. In another experiment, mice were treated weekly with anti-I-J antibody, dialyzable leukocyte extract, or saline from 18 months of age until natural death. The mice were immunized with avian gammaglobulin at 27 and again at 29 months of age. Both types of immunologic intervention resulted in a greater secondary antibody response than that of the saline-treated control mice. Mice treated with anti-I–J antibody survived longer than did mice of the other two groups. There was a correlation between the magnitude of the secondary response of individual mice and their lifespan. The results provide support for the immunologic theory of aging.

Key words: Dialyzable leukocyte extract; Anti-I–J antibody; Immunologic theory of aging; Lymphocyte function; humoral immune response

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INTRODUCTION

One of the characteristics of aging is the decline in the ability of an individual to adapt to environmental stress. Consequently, there is an increase in the incidence of so-called "diseases of aging", such as late-occurring neoplasia, arterial diseases, renal diseases, autoimmune and immune complex diseases. The immune system is also affected by advancing age. All aspects of the immune response, including humoral and cell-mediated responses (reviewed in ref. 1), and extending to some aspects of natural resistance [2], are affected by the aging process.

The immune system is entrusted with the preservation of the integrity of organs and tissues. It is possible, therefore, that the age-related dysfunction of the immune system may be involved in the pathogenesis of the lesions of aging and "diseases of aging". This so-called "immunologic theory of aging" was advanced by Walford in 1969 [3]. Though direct evidence of the validity of this theory is lacking, certain observations are at least consistent with it. Many of the so-called diseases of aging involve a deterioration of the immune system. Lifespan in mice is correlated with the major histocompatibility complex, the master gene of the immune system [4]. In addition, regimens that have been shown to increase longevity, such as calorie restriction [5] and lowering of body temperature [6], also result in a delay in the age-related decline of immune function [7,8]. While these findings suggest that there is a relation between longevity and immunologic vigor in old age, they do not establish that the latter is the cause of the former. We therefore initiated experiments to test whether immunologic interventions intended to maintain the integrity of the immune system could delay the onset of the diseases of aging and prolong lifespan.

Earlier findings from this laboratory demonstrated an age-related increase in suppressor activity in mice, which occurs at a time (prior to 15 months of age) when T-helper and B-cell functions are still relatively vigorous [9]. This suggests that specific inhibition of T-suppressor cells at this time may be a valid approach to the rejuvenation of the immune system. Since T-suppressor cells [10,11] and derived suppressor factors [12] express the antigenic determinants coded for by the I–J subregion of the H-2 gene complex, they may be expected to be selectively inactivated or reduced in numbers by antibody directed against these determinants. This notion is strengthened by reports that *in vivo* administration of anti-I–J alloantiserum inhibited sarcoma growth in A/J mice [13] and increased by 2–3-fold the plaque-forming cell (PFC) response to suboptimal doses of sheep red blood cells (SRBC) [14].

Human dialyzable leukocyte extract (DLE), containing transfer factor (TF), has been used as an immunoprophylactic and/or immunotherapeutic agent (reviewed in ref. 15). Although the precise mechanism of action of DLE is still unknown, both antigen-specific effects and nonspecific effects have been reported. The antigen-specific effect, *i.e.* TF activity of DLE, is indicated by the ability to transfer delayed-type hypersensitivity and cell-mediated immune reactivity [16]. The nonspecific effects of DLE include increased chemotactic activity for monocytes and neutrophils [17], increase in con-

centration of cyclic nucleotides in monocytes [18], enhancement of antibody production [19], and enhancement of T and B mitogen responses [20].

In view of the findings summarized above, we decided to study the effects of anti-I–J monoclonal antibody and of DLE on the immune potential of aged mice and on their lifespan. Several experiments were conducted; however, a clear-cut effect of immunotherapy on immune potential was demonstrated in only one of them. In that same experiment, the immunotherapeutic intervention appeared to prolong the lifespan of the experimental mice.

MATERIALS AND METHODS

Mice

Female (C57BL × C3H) F1 (BC3F1) mice were purchased from Cumberland View Farms, Clinton, TN. This mouse strain has a median lifespan of approximately 30 months. Random-bred female retired breeders of the ICR strain (Harlan Industries, Indianapolis, IN) were used as recipients of Millipore diffusion chambers.

Immunotherapies

Monoclonal anti-I–J^b (WF9.40.5) and anti-I–J^k (WF8.C12.8) antibodies were received as sterile ascites fluid from Northwestern University Medical School in Chicago [21]. A mixture containing equal portions of anti-I–J^b and anti-I–J^k monoclonal antibodies was diluted in pyrogen-free saline. DLE of human origin was prepared at the Medical University of South Carolina in Charleston. It has been shown that DLE has both nonspecific and specific (transfer factor) activities in species other than that in which it is generated [22]. Two "blind" preparations identified only by code letters were used. One of the preparations contained DLE and the other contained only the saline diluent.

Immunogen

Avian gamma-globulin (AGG) was prepared by 50% saturated ammonium sulfate precipitation of pooled chicken sera and further purified by column chromatography on DEAE-cellulose. AGG was adsorbed on bentonite [23].

Experimental protocol

In the first experiment, we investigated the effects of long-term treatment with anti-I–J or with DLE on lymphocyte function. Three groups of ten 14-month-old BC3F1 mice were each treated with 1 ml of anti-I–J monoclonal antibody diluted 1:50, or 1 ml of DLE diluted 1:10, or 1 ml of saline, once a week for 1 year. At the end of the treatment, the mice were killed and their spleen cells were assayed for suppressor, T-helper and B-cell function.

The assays were described in a previous paper [9]. Briefly, single-cell suspensions from the spleen of individual mice were divided into two portions. One portion was tested for suppressor activity by adding the cells to be tested to spleen cells of young

syngenic mice that had been primed with dinitrophenylated human gamma-globulin (DNP-HGG), boosting the cell mixture with DNP-HGG, propagating it in Millipore diffusion chambers implanted in recipient mice, and enumerating the DNP-specific PFC 1 week later. The percentage suppression was calculated by comparison with cultures to which test cells had not been added. The second portion of the spleen cell suspension was passed through a column of nylon wool. Immune complexes made with keyhole limpet hemocyanin (KLH) and anti-KLH antibodies were added to the nonadherent cells, which were then propagated in diffusion chambers. Six days later, the cells were recovered and assayed for carrier (KLH)-specific helper activity by adding them to T-cell-depleted (by treatment with monoclonal anti-Thy-1.2 antibody and complement) spleen cells from young mice that had been primed with DNP-HGG. The cell mixture was boosted with DNP-KLH, propagated in diffusion chambers, and assayed for DNP-specific PFC 1 week later. The ratio of the number of PFC found in cultures containing both carrier-primed and hapten-primed cells to that obtained in cultures containing only hapten-primed cells (enhancement ratio) was taken as a measure of T-helper activity in the test cells. The cells that adhered to the nylon wool were recovered, immunized *in vitro* with the T-independent antigen DNP-Ficoll, propagated in diffusion chambers, and assayed for DNP-specific PFC to measure the B-cell activity. Organs and small pieces of spleen were processed for histopathologic study. A group of six 14- to 21-week-old untreated BC3F1 mice was used as control.

The second experiment was designed to measure the effects of long-term administration of anti-I–J and DLE on both lifespan and on immune responsiveness to the T-dependent antigen AGG. Three groups of eight 18-month-old BC3F1 mice were treated weekly with intraperitoneal injections of 1 ml of anti-I-J, DLE, or saline. The treatment was continued until death of these mice. Nine months after the initial treatment, the mice were primed with 100 μg of AGG adsorbed on bentonite and challenged with the same antigen 1.5 months later. A group of four untreated 7-month-old BC3F1 mice was primed and challenged at the same time. Sera were collected 1 week after each immunization and titrated by both tannic acid hemagglutination (HA) [24] and enzyme-linked immunosorbent assay (ELISA) [25]. Titers were expressed as the $\log_2$ of the reciprocal of the end-point dilution of serum. In the ELISA, the end-point was the highest dilution of the test serum with adsorbance at least equal to 50% of the mean maximal adsorbance given by a pool of sera from immune young mice that was titrated with each ELISA plate. Separate HA titrations were done with each test serum for total and 2-mercaptoethanol (ME)-resistant (IgG) antibodies. In the ELISA, IgM- and IgG-specific enzyme-linked antibodies (Zymed Laboratories, San Francisco, CA) were used in separate titrations.

Statistical analysis

A two-tailed Student's *t*-test was used to determine differences between groups. Differences were considered statistically significant when the p value was 0.05 or less. Regression analysis was used to calculate regression lines and correlation coefficients.

RESULTS

Effects of long-term treatment with anti-I–J, DLE, or saline on lymphocyte function

Suppressor activity of the test spleen cells (TC) derived from individual mice treated weekly from 14 to 26 months of age was measured by the reduction of the secondary anti-DNP PFC response of mixed cultures, containing equal numbers of reference primed cells (RC) and TC, compared to that of RC only. The effects of the treatments on suppressor activity, presented in Table I, show that spleen cells from young untreated mice did not have any significant suppressor activity. In contrast, the spleen cells of 26-month-old saline-treated mice (group III) were highly suppressive, indicating an age-related increase of suppressor activity. Treatment with DLE (group II) significantly decreased the suppressor activity. There was no significant difference in suppressor activity between anti-I–J-treated (group I) and saline-treated (group III) mice.

T-helper (Th) cell activity was assessed by measuring the ability of T cells activated with carrier–anti-carrier immune complexes [26] to restore the antibody response of hapten-primed, T-depleted syngeneic cells. The ratio of PFC produced by the cultures containing the test Th cells and DNP-primed B cells to the PFC found in cultures containing only primed B cells (enhancement ratio) was taken as a measure of the extent of Th cell function. The results are shown in Table II. The Th function of saline-treated old mice (group III) was only one-fifteenth of that of young mice (group IV). This difference was highly significant. Treatment with anti-I–J (group I) partially prevented the age-related loss of the Th activity, but there was no statistically significant difference between this group and the saline-treated control mice (group III).

TABLE I

EFFECT OF ANTI-I–J, DLE, AND SALINE TREATMENT ON SUPPRESSOR ACTIVITY

Group	*Treatment*	*No. of mice*	*Mean PFC/culture*		*Mean percentage suppression*[c]
			RC[a]	*RC + TC*[b]	
I	Anti-I–J	8	121 307	52 736	56.4
II	DLE	7	86 670	50 153	39.5*
III	Saline	8	109 807	36 984	66.4
IV	Untreated (young)	6	121 051	105 043	30.4**

*Significantly different from group III ($p < 0.05$).

**Significantly different from group III ($p < 0.01$).

[a] 15×10^6 reference cells (spleen cells from young-adult mice that had been primed 3–5 weeks earlier with DNP-KLH).

[b] 15×10^6 test cells (spleen cells from the mice being tested for suppressor activity).

[c] Means of the percentage suppression values of individual mice in each group. Percentage suppression for each mouse was calculated by dividing the mean number of PFC of at least three cultures containing RC + TC by the mean number of PFC of the control cultures for the experiment, subtracting the resulting figure from 1, and multiplying the result by 100.

TABLE II

EFFECT OF ANTI-I–J, DLE, AND SALINE TREATMENT ON T-HELPER ACTIVITY

Group	*Treatment*	*No. of mice*	*Mean PFC/culture*		
			Th[a] + *B*[b]	*B*	*Th + B/B*[c]
I	Anti-I–J	6	5 172	414	6.1
II	DLE	3	384	278	1.9
III	Saline	7	2 601	431	2.8
IV	Untreated (young)	5	17 712	454	40.2*

*Significantly different from group III ($p < 0.05$).

[a] 1.5×10^6 cells obtained from diffusion chambers seeded 6 days earlier with nylon wool-enriched T-cells from a test mouse, stimulated with KLH–anti-KLH immune complexes.

[b] 15×10^6 anti-Thy-1.2- and C-treated (T-depleted) spleen cells from young adult mice primed 3–5 weeks earlier with DNP-HGG.

[c] Mean of the enhancement ratios of individual mice in each group. Enhancement ratio for each mouse was the mean number of PFC of at least three cultures containing Th cells from that mouse + B-cells, divided by the mean number of PFC of cultures containing B-cells in that experiment only.

B-cell activity was assessed by measuring the primary response to the T-independent antigen DNP-Ficoll. The results are shown in Table III. No significant differences were found among the four groups of mice, but it is difficult to draw any conclusion from this study, since the PFC response found in young mice was much less than what we had found in previous experiments [9] using mice of comparable age.

There were no significant differences in tumor incidence or in other histopathologic findings among treated groups. No tumors were observed in any of the control young mice. However, seven of nine saline-treated old mice had malignant tumors which were classified as either lymphosarcoma or reticulum cell sarcoma. Four of eight anti-I–J-treated mice and four of seven DLE-treated mice had similar types of tumors.

TABLE III

EFFECT OF ANTI-I–J, DLE, AND SALINE TREATMENT ON B-CELL ACTIVITY

Group	*Treatment*	*No. of mice*	*Mean PFC/culture (S.E.M.)*[a]
I	Anti-I–J	8	390 (± 71)
II	DLE	7	1478 (± 475)
III	Saline	8	3086 (± 552)
IV	Untreated (young)	6	3218 (± 757)

[a] 15×10^6 nylon wool-enriched B-cells were cultured with 50 ng of DNP-Ficoll in a diffusion chamber for 7 to 8 days. The B-cell function of each individual mouse was expressed as the mean direct anti-DNP PFC response of at least three cultures.

Effects of long-term treatment with anti-I–J, DLE, or saline on the humoral immune response and on lifespan

Three groups of eight 18-month-old mice were injected weekly with anti-I–J monoclonal antibody, DLE, or saline, respectively, until natural death. The mice were immunized with AGG at 27 and again at 29 months of age. Seven days after each immunization, the mice were bled and their sera were assayed by HA and ELISA for anti-AGG antibodies.

The mean serum HA titers against AGG are shown in Table IV. The primary immune response of saline-, anti-I–J, or DLE-treated old mice was almost as good as that of young mice in both total and 2-ME-resistant titer. In contrast, saline-treated old mice (group III) responded much less than young mice (group IV) did in the secondary immune response. Both anti-I–J and DLE treatments enhanced the secondary mean total and 2-ME-resistant serum titer in old mice. The difference between groups I and III was significant. In fact, the response of anti-I–J-treated old mice (group I) was not significantly different from that of young control mice.

Similar results were found with ELISA, as indicated in Table V. No significant age-related decline was found in primary IgG and IgM titers. However, both secondary IgM and IgG titers of saline-treated old mice (group III) were significantly lower than those of young mice (group IV). Treatment with anti-I–J enhanced the secondary IgG, but not the IgM response.

The lifespan of mice treated with anti-I–J, DLE, or saline is shown in Table VI. The mean lifespan of anti-I–J-treated mice was prolonged by 12% over that of saline-treated controls, the median lifespan was 8% greater than that of control mice, and the last survivor in the anti-I–J group lived 16% longer than the last survivor in the saline-treated control group. Treatment with DLE had no apparent effect on lifespan. The probability that the difference in longevity between the anti-I–J and saline-treated groups was due to chance alone was 0.07. Since the mean survival time of DLE-treated and saline-treated

TABLE IV

HEMAGGLUTINATION TITERS OF AGED MICE TREATED WITH ANTI-I–J, DLE, OR SALINE

Group	*Treatment*	*Mean $\log_2$ titer (No. of mice)*			
		Primary		*Secondary*	
		Total	*2-ME-resistant*	*Total*	*2-ME-resistant*
I	Anti-I–J	12.5(6)	8.3(6)	18.6**(5)	18.0**(5)
II	DLE	13.0(5)	8.6(5)	17.0(3)	17.0(3)
III	Saline	13.3(4)	7.0(4)	14.3(3)	15.0(3)
IV	Untreated (young)	13.3(4)	9.5*(4)	19.0***(4)	19.5***(4)

*Significantly different from group III ($p < 0.01$).
**Significantly different from Group III ($p < 0.001$), but not from group IV.
***Significantly different from group III ($p < 0.005$), but not from group I.

TABLE V

ELISA TITERS OF AGED MICE TREATED WITH ANTI-I–J, DLE, OR SALINE

Group	*Treatment*	*Mean $\log_2$ titer (No. of mice)*			
		Primary		*Secondary*	
		IgM	*IgG*	*IgM*	*IgG*
I	Anti-I–J	9.0(6)	14.5(6)	6.2(5)	19.4**(5)
II	DLE	9.0(5)	14.8(5)	5.1(1)	17.6(3)
III	Saline	8.9(4)	14.5(4)	5.4(3)	16.9(3)
IV	Untreated (young)	9.7(4)	14.6(4)	9.9*(4)	19.2***(4)

*Significantly different from group III ($p < 0.001$).
**Significantly different from group III ($p < 0.01$), but not from group IV.
***Significantly different from group III ($p < 0.05$), but not from group I.

control mice was almost identical (Table VI), the data from these two groups were pooled and the statistical significance of the difference between the mean lifespan of the pooled groups and the mean lifespan of the anti-I–J-treated mice was tested. When this was done, the difference was found to be significant ($p < 0.05$).

The survival curves of the anti-I–J-, DLE-, and saline-treated groups of mice are shown in Fig. 1. The survival curve of anti-I–J-treated mice shifted to the right of that of control mice.

The relationship between the lifespan of individual mice and their total secondary HA titer to AGG is shown graphically in Fig. 2. There was a significant ($p < 0.02$) positive linear correlation between lifespan and total secondary HA titer.

Two additional experiments were conducted in which groups of mice 21–22 and 18.5 months of age, respectively, were treated with weekly injections of anti-I–J monoclonal antibody or saline until the time of death. All the mice were immunized with

TABLE VI

LIFESPAN OF MICE TREATED WITH ANTI-I–J, DLE, OR SALINE

Group	*Treatment*	*No. of mice*[a]	*Weeks*		
			Mean	*Median*	*Maximum*
I	Anti-I–J	8	132.5	129.0	158
II	DLE	8	117.1	121.5	145
III	Saline	8	117.9*	119.5	135

*$p = 0.07$ *vs.* group I; $p = 0.93$ *vs.* group II.
[a]The mice in this experiment belonged to the same cohort. No data on mortality were collected prior to the beginning of the experimental treatments at 18 months of age.

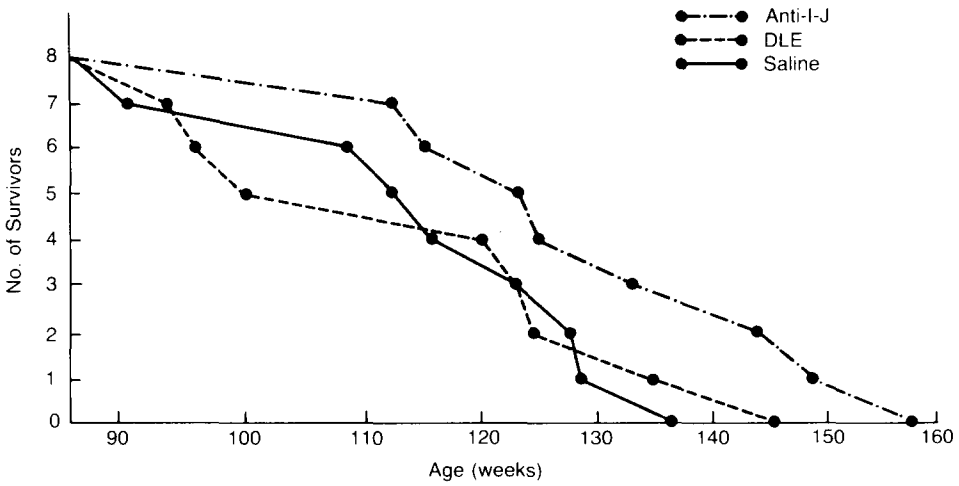

Fig. 1. Survival curves of mice treated weekly with anti-I–J antibody, DLE, or saline from 18 months of age until natural death.

AGG at 26 and at 27.5 months of age and their antibody titers were measured 1 week after each immunization. In neither of these experiments was a difference in the magnitude of the immune response detected between the saline-treated and anti-I–J-treated mice, nor was there a difference in longevity (results not shown).

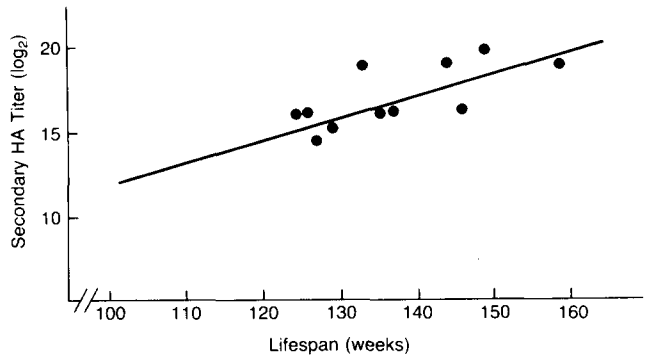

Fig. 2. Correlation between the log_2 secondary HA titer of aged mice and their age at death. The mice were immunized with avian gamma-globulin at 27 and again at 29 months of age. Sera were obtained 1 week after the second immunization. Serum titers of mice of all three treatment groups are included in the plot. The correlation coefficient was 0.69; the correlation was statistically significant ($p. < 0.02$).

DISCUSSION

The goal of gerontologic research is not only to understand the mechanisms which cause aging, but also to search for methods to delay the onset or lessen the severity of the diseases associated with aging, thus prolonging the productive period of life. Although our knowledge of the aging process is still quite limited, certain methods have been successful in prolonging lifespan. These include genetic manipulation [27] and splenectomy [28] in mice, caloric restriction in mice and rats [5,29], and lowering of body temperature in fish [8]. All these manipulations also had some positive effects on the immune system.

It has long been known that immune responsiveness declines in an age-related fashion after sexual maturity, in parallel with the aging process [1]. Because the loss of immunologic vigor appears to be causally related to the increased susceptibility to "diseases of aging", such as certain types of infectious diseases, autoimmune and immune complex diseases, and cancer, there has been a growing interest in searching for methods of intervention on the immune system. These immunologic interventions include cell grafting [30] and chemical therapy, the latter consisting of administration of synthetic polynucleotides [31], levamisole [32], thymic factors [33] and 2-mercaptoethanol [34]. Although all of these immunomodulators possess certain immunorestorative activities, none of them has been reported to have any effect on age-related disease pattern and on lifespan.

In this study, we have attempted to devise a critical test of the immunologic theory of aging. Our rationale is that, if the cause–effect relationship between dysfunction of the immune system and pathogenesis of the "diseases of aging" holds true, as proposed by Walford, then immunologic interventions which prevent the age-related dysfunction of the immune system should be able to delay the onset of the diseases of aging and prolong lifespan.

It was important that the treatments chosen affect exclusively or primarily the immune system. We chose anti-I–J antibody because the I–J determinant is present only on lymphocytes of the suppressor circuit and on the suppressor factors they produce [35], and DLE because it is a lymphocyte product, although its activity affects other cells as well [17,18]. Administration of these materials was commenced when the mice were adult, and it was continued through old age, on the assumption that a treatment limited in time could not be expected to arrest the progressive and continuous deterioration of the immune system which accompanies the aging process. The parameters examined included the magnitude of the humoral antibody response, which should reflect the overall function of the immune system; the activity of the suppressor, T-helper and B-cells, whose function we had shown to decline in an age-related fashion, but at different rates [9]; and longevity, which, if affected by the experimental treatments, would establish the connection between the immune system and the aging process.

The results of measurements of lymphocyte activity were not very informative. The finding of an increase in suppressor activity accompanying a decline of T-helper activity

in saline-treated old mice is in agreement with our earlier findings [9]. Treatment with anti-I–J monoclonal antibody had no effect on suppressor activity, but appeared to increase the activation of carrier-specific T-helper cells, although the difference between anti-I–J-treated and saline-treated mice was not statistically significant. Since the helper cells is the ultimate target of the suppressor circuit [35], an increase in T-helper activity is also compatible with the postulated effect of anti-I–J antibody on I–J-positive suppressor cells. Treatment with DLE significantly decreased the nonspecific suppressor activity of cells from old mice, whereas no effect was noticed on the T-helper activity. With either treatment, no conclusion could be derived from measurements of B-cell activity, because the results were exceedingly variable and were made unreliable by technical difficulties experienced in these experiments. Similarly, although a lower incidence of tumors was recorded in the treated mice than in the saline-treated controls, the small numbers of animals used did not permit an assessment of the effects of the treatments on late-occurring tumors.

More interesting results were obtained in one of the experiments designed to measure the effects of immunotherapy on the magnitude of the immune response and on lifespan. The secondary humoral response of anti-I–J-treated mice, which was greater than that of saline-treated controls and reached the same level as that of young untreated mice, indicates that the immune system of anti-I–J-treated mice was functioning with youthful efficiency in old age. The secondary response of DLE-treated mice was also greater than that of control mice, but the difference did not reach a statistically significant level.

Treatment with anti-I–J antibody also appeared to prolong lifespan in the experimental mice. Both median and maximal lifespan were extended, suggesting that the effect was not due exclusively to a lower incidence of disease in the experimental group, which would be expected to affect the median, but not the maximal, lifespan. Rather, comparison of the survival curves of anti-I–J-treated and saline-treated control mice suggests that the aging process itself proceeded at a slower rate in the experimental mice. Since the target of anti-I–J antibody presumably is the immune system, the results provide support for Walford's immunologic theory of aging.

The difference in mean lifespan between the anti-I–J- and the saline-treated groups approached, but did not reach, the level of statistical significance commonly accepted in biological experiments. Attempts to reproduce the results were unsuccessful. Thus, it is possible that the results obtained were due to chance and not to the treatment with anti-I–J antibody. We believe, however, that it is significant that in the experiment in which treatment increased the immune potential of the aged mice, prolonged lifespan was also observed. In subsequent experiments, immunotherapy was not effective insofar as it did not affect the immune potential of the aged mice; there was no prolongation of lifespan in these experiments. The variable effects of immunotherapy may be related to the degree of the age-associated deterioration of the immune system of the mice at the beginning of treatment, to the strength of the unknown stimulus that results in the generation of suppressor cells with advancing age [36], to the state of other elements of the immune system that were not specific targets of the experimental immunologic inter-

vention, or to other unknown and uncontrolled variables. Regardless of the cause for the inconsistent effects of administration of anti-I–J antibody, we conclude that *successful* immunotherapy resulted in increased longevity.

This conclusion is strengthened by finding that there was a correlation between the secondary antibody titer of mice immunized at 27 and 29 months of age and their longevity. Thus, the animals which, for whatever reason, had maintained a vigorous immune system in old age lived longer than those whose immune potential had declined. In the present work, measurement of immune potential was a predictor of longevity. Ludwig and Masoro [37] point out that while gerontological literature "abounds with reports on age dependence of functional loss, reserve capacity, or structural change... correlations between age-related changes and viability of the organisms that exhibit them" are lacking. When such correlations have been sought, they have generally not been found [38]. It is possible, therefore, that immune potential may be a useful marker of biological age. It must be pointed out, however, that in this work immune potential was measured in aged mice which had already experienced a considerable loss of immune function. Measurement of immune potential at an earlier age may not be correlated with lifespan, especially since the age-related functional decline of the various components of the immune system does not always proceed at a constant rate [9]. In any case, the finding of a correlation between secondary antibody response and longevity is reminiscent of the observation by Roberts-Thomson *et al.* [39] that elderly people who were hyporesponsive in skin tests for delayed hypersensitivity were at greater risk of death than people of the same age who responded normally to skin tests. Both observations are consistent with a cause–effect relation between the deterioration of the immune system and aging.

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REFERENCES

1 T. Makinodan and M.M.B. Kay, Age influence on the immune system. *Adv. Immunol., 29* (1980) 287–331.
2 R. Kiesling and O. Haller, Natural killer cells in the mouse: An alternative immune surveillance mechanism. *Contemp. Topics Immunobiol., 8* (1978) 171–201.
3 R.L. Walford, *The Immunologic Theory of Aging,* Munksgaard, Copenhagen, 1969.
4 R.M. Williams, L.J. Kraus, P.T. Lavin, L.L. Steele and E.J. Yunis, Genetics of survival in mice: Localization of dominant effects to subregions of the major histocompatibility complex. In D. Segre and L. Smith (eds.), *Immunological Aspects of Aging,* Marcel Dekker, New York, 1981, pp. 247–353.
5 C.M. McCay, M.F. Crowell and L.A. Maynard, The effect of retarded growth upon the length of lifespan and upon the ultimate body size. *J. Nutr., 10 (1935)* 63–79.

6 R.L. Walford and R.K. Liu, Husbandry, life span, and growth rate of the annual fish *Cynolebias adolffi. Exp. Gerontol., 1* (1965) 161–171.

7 R.H. Weindruch, J.A. Kristie, K.E. Cheney and R.L. Walford, Influence of controlled dietary restriction on immunologic function and aging. *Fed. Proc., 38* (1979) 2007–2016.

8 R.K. Liu and R.L. Walford, The effect of lowered body temperature on lifespan and immune and non-immune processes. *Gerontolgia, 18* (1972) 363–388.

9 J.J. Liu, M. Segre and D. Segre, Changes in suppressor, helper, and B-cell functions in aging mice. *Cell. Immunol., 66* (1982) 372–382.

10 D.B. Murphy, L.A. Herzenberg, K. Okumura and H.O. McDevitt, A new I subregion (I–J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. *J. Exp. Med., 144* (1976) 699–712.

11 O. Primi, L. Hammarstrom, E. Moller and C.I.R. Smith, Genetic control of effector cell characteristics: Con A-induced suppressor cells carry H-2 I region encoded determinants. *Cell. Immunol., 47* (1979) 143–152.

12 J. Theze, C. Waltenbaugh, M.E. Dorf and B.J. Benacerraf, Immunosuppressive factor(s) specific for L-glutamic acid^{50}-L-tyrosine^{50} (GT). II. Presence of I–J determinants on the GT-suppressive factor. *J. Exp. Med., 146* (1977) 287–292.

13 M.I. Greene, M.E. Dorf, M. Pierres and B. Benacerraf, Reduction of syngeneic tumor growth by an anti-I–J alloantiserum. *Proc. Natl. Acad. Sci. USA, 74* (1977) 5118–5121.

14 M. Pierres, R.N. Germain, M.E. Dorf and B. Benacerraf, Potentiation of a primary *in vivo* antibody response by alloantisera against gene products of the I region of the H-2 complex. *Proc. Natl. Acad. Sci. USA, 74* (1977) 3975–3979.

15 P.H. Klesius, H.H. Fudenberg and C.L. Smith, Comparative studies on dialyzable leukocyte extracts containing transfer factor–A review. *Comp. Immunol. Microbiol. Infect. Dis., 3* (1980) 247–260.

16 D. Rifkind, J.A. Frey, E.A. Peterson and M. Dinowitz, Transfer of DH in mice to microbial antigens with dialyzable transfer factor. *Infect. Immun., 16* (1977) 258–262.

17 J.L. Galli and C.H. Kirkpatrick, Chemotactic activity in dialyzable transfer factor. *Proc. Natl. Acad. Sci. USA, 71* (1974) 498–502.

18 J.A. Sanders, T.K. Smith, V.C. Manganicello and C.H. Kirkpatrick, Stimulation of monocyte cGMP leukocyte dialysates: An antigen-independent property of dialyzable transfer factor. *J. Clin. Invest., 56* (1975) 1271–1279.

19 M.R. Salaman, I.L. Sargent and V.S. Slijivic, Transfer factor as an adjuvant for antibody production in the mouse. In A. Khan, C.H. Kirkpatrick and N.O. Hill (eds.), *Immune Regulators in Transfer Factor,* Academic Press, New York, 1979, pp. 661–665.

20 T.K. Huard, Effects of the injection of human dialyzable leukocyte lysate (HDLL) fractions into mice on subsequent responses to mitogens. In A. Khan, C.H. Kirkpatrick and N.O. Hill (eds.), *Immune Regulators in Transfer Factor,* Academic Press, New York, 1979, pp. 633–649.

21 C.R. Waltenbaugh, Regulation of the immune response by I–J gene products. I. Production and characterization of anti-I–J monoclonal antibodies. *J. Exp. Med., 154* (1981) 1570–1583.

22 H.H. Fudenberg and K.Y. Tsang, *In utero* osteosarcoma tolerized hamsters: A model for human cancer and immunocyte differentiation. In *Nobel Symposium: First International Conference on Progress in Cancer Research,* in press.

23 M.B. Rittenberg and K.L. Pratt, Anti-trinitrophenyl (TNP) plaque assay. Primary responses of Balb/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med., 132* (1969) 575–581.

24 A.B. Stavitsky, Hemagglutination with tannic acid-treated (tanned) erythrocytes. In C.A. Williams and M.W. Chase (eds.), *Methods in Immunology and Immunochemistry,* Vol. 4, Academic Press, New York, 1977, pp. 30–41.

25 A. Voller, D.E. Bidwell and A. Bartlett, Enzyme immunoassays in diagnostic medicine. Theory and Practice. *Bull. WHO,* 53 (1976) 55–65.

26 D.-H. Ding, M. Segre and D. Segre, Activation of helper T cells by immune complexes. *Cell. Immunol., 46* (1977) 281–296.

27 G.S. Smith and R.L. Walford, Influence of the main histocompatibility complex on aging in mice. *Nature, 46* (1977) 727–729.

28 J.F. Albright, T. Makinodan and J.W. Deitchman, Presence of life-shortening factors in spleens of aged mice of long lifespan and extension of life expectancy by splenectomy. *Exp. Gerontol., 4* (1969) 267–276.
29 R.H. Weindruch, J.A. Kristie, H. Naeim, B.G. Mullen and R.L. Walford, Influence on weaning-initiated dietary restriction on responses to T cell mitogens and on splenic T cell levels in a long-lived Fl-hybrid mouse strain. *Exp. Gerontol., 17* (1982) 49–64.
30 R.L. Walford, P.J. Meredith and K.E. Cheney, Immunoengineering: Prospects for correction of age-related immunodeficiency states. In T. Makinodan and E. Yunis (eds.), *Immunology of Aging,* Plenum, New York, 1977, pp. 183–201.
31 W. Braun, Y. Yazima and M. Ishizuka, Synthetic polynucleotides as restorers of normal antibody formation capacities in aging mice. *J. Reticuloendoth. Soc., 7* (1970) 418–424.
32 J.W. Hodden, R.G. Coffey, E.M. Hadden, E. Lopez-Corrales and G.H. Sunshine, Effect of levamisole and imidazole on lymphocyte proliferation and cyclic nucleotide levels. *Cell. Immunol., 20* (1978) 98–103.
33 A.L. Goldstein, G.B. Thurman, T.L.K. Low, G.E. Trivers and J.L. Rossio, Thymosin: The endocrine thymus and its role in the aging process. In A. Cherkin, C.E. Finch, N. Kharasch, T. Makinodan, F.L. Scott and B.L. Strehler (eds.), *Physiology and Cell Biology of Aging,* Raven Press, New York, 1979, pp. 51–60.
34 M.P. Chang, J.L. Tanaka, S. Stosic-Grujicic, E.K. Yamamoto, E.H. Perkins, B.L. Strehler and T. Makinodan, Restoration of impaired immune functions in aging animals. VI. Differential potentiating effect of 2-mercaptoethanol on young and old immune spleen cells. *Int. J. Immunopharmacol., 4* (1982) 429–436.
35 D.R. Green, P.M. Flood and R.K. Gershon, Immunoregulatory T-cell pathways. *Annu. Rev. Immunol., 1* (1983) 439–463.
36 D. Segre and M. Segre, Humoral immunity in aged mice. II. Increased suppressor T cell activity in immunologically deficient old mice. *J. Immunol., 116* (1976) 735–738.
37 F.C. Ludwig and E.J. Masoro, The measurement of biological age. *Exp. Aging Res., 9* (1983) 219–220.
38 D.E. Harrison and J.R. Archer, Physiological assays for biological age in mice: Relationship of collagen, renal function, and longevity. *Exp. Aging Res., 9* (1983) 245–251.
39 I.C. Roberts-Thomson, S. Whittingham, V. Youngchaiyud and I.R. MacKay, Aging, immune response, and mortality. *Lancet, ii* (1974) 368–370.