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INFLUENCES OF SODIUM DIETHYLDITHIOCARBAMATE, DTC (IMUTHIOL[R]) ON T CELL DEFECTIVE RESPONSES OF AGED BALB/c MICE

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Abstract — The effect of chronic imuthiol treatment, 25 mg/kg weekly for 4 months initiated at the age of 12 months, on T-cell functions of aged female BALB/c mice was investigated. Imuthiol restored to normal value the impaired response to Concanavalin A (Con A) and enhanced the proliferative response to phytohemagglutinin (PHA). Impaired cytotoxic T-cell activity (CTL), was restored near to the value of young controls by imuthiol. Serum thymic factor (FTS) levels in serum of treated aged animals outpassed those of untreated young mice. Delayed-type hypersensitivity (DTH) reaction to oxazolone was increased. In contrast, the graft-vs-host (GVH) mortality induced by injecting H-2 histoincompatible cells to irradiated recipients, which GVH was impaired by aging, was not significantly modified by imuthiol. The excessive cytotoxicity for chicken red cells of macrophages (ADCC) from aged mice was reduced, as well as macrophage cytotoxicity for tumor cells. Natural killer cell activity remained unchanged. This finding confirms that imuthiol enhanced effectively T cell-dependent responses but the data on GVH reaction suggest that its effects are under a complex mode of action. Restoration of a normal production of FTS may be one mechanism by which imuthiol acts on the reinduction of the T-cell differentiating pathway in aged mice.

Correction of immunological senescence is an attractive area of research. Thymus atrophy (Hirokawa and Makinodan, 1975), progressive disappearance of thymic hormones (Bach, Dardenne, Pleau & Bach, 1975) and decline of T cell reactivity (Makinodan & Kay, 1980) due to reduced T cell help (Krogsrud & Perkins, 1977) or increased T cell suppression (Segre & Segre, 1976), are associated with aging. This depression of T cell functions has been in part ascribed to impairment of IL-2 production by helper T cells (Thoman & Weigle, 1981; Miller & Stutman, 1981). Thymus grafts and thymic extracts have been employed with transient and limited successes. T cell stimulating compounds, such as bestatin or tuftsin, were able to restore some of the T cell responses, delay mortality and reduce or delay the development of tumors (Bruley-Rosset, Florentin, Kiger, Schulz & Mathé, 1979; Bruley-Rosset, Hercend, Martinez, Rappaport & Mathé, 1981). Those agents were able to activate not only T cells but also other cells effective in immunity as macrophages.

We selected for the present work, a synthetic chemically-defined compound, sodium diethyldithiocarbamate, DTC (imuthiol[R]), which displays a specific influence on the T-cell lineage (Renoux, 1982; Renoux & Renoux, 1977). Moreover, this compound was shown to stimulate immune responses with identical magnitude, as well after a chronic than after a single administration (Renoux & Renoux, 1981a). It was demonstrated to be also active in congenitally athymic mice (Renoux & Renoux, 1983; Renoux, Renoux, Guillaumin & Gouzien, 1979) or in syndromes where a T-cell dysfunction was apparent. It appeared therefore that imuthiol was a good candidate for the restoration of the immune deficiency observed in aged mice.

EXPERIMENTAL PROCEDURES

Mice

Inbred female BALB/c mice were purchased from BOM Holtgaard Laboratories (Denmark) at 6–8

weeks of age and kept in an air filtered and conditioned room.

Drug and treatment

Lyophilized sodium diethyldithiocarbamate (DTC, now imuthiol[R]) was obtained from Institut Mérieux (Lyon, France), and dissolved in a pyrogen-free saline buffer, pH 7.2. Fifty mice were treated by a weekly intraperitoneal injection of 25 mg/kg imuthiol at the age of 12 months until 16 months. A control group consisted of 60 saline-treated mice. Five days after the last injection, modifications of immunological reactivity was assessed by *in vitro* and *in vivo* testing in imuthiol-treated and saline-treated 16-month-old aged mice and compared with 3-month-old untreated female BALB/c mice (young control group).

Cell preparation

Spleen and peritoneal exudate cells were obtained from 4 to 5 mice and pooled to perform the various immunological tests. Spleen cells were removed aseptically and single cell suspensions were prepared in RPMI 1640 medium. Viability was determined by trypan blue dye exclusion. Peritoneum were carefully washed with Hank's balanced solution and the number of macrophages was estimated by neutral red uptake.

Spleen cell response to T cell mitogens

Mitogenic response was assessed in triplicate wells in microplates (Falcon) containing 5×10^5 cells in 0.2 ml of RPMI 1640 medium supplemented with 5% heat-inactivated mule serum and antibiotics in the presence of an optimal concentration of phytohemagglutinin (PHA; Wellcome Laboratories, 1:50 dilution) or concanavalin A (Con A; pharmacia, 1 μg/ml). After 48 h of incubation at 37°C, cultures were pulsed with 1 μCi (^{3}H)-thymidine per well, and cells were collected 6 h later. Three experiments using a pool of spleen cells derived from 3 mice of each group were performed.

Cytotoxic T cell induction (CTL)

Pooled spleen cells from 4 to 5 BALB/c mice (5×10^6 per well of a multiwell plate, Linbro) were incubated in the absence (control group) or presence (experimental group) of 5×10^4 mitomycin-C-treated EL4 lymphoma cells. The RPMI 1640 medium contained antibiotics, glutamine and 5×10^{-5} M 2-mercaptoethanol (2-ME). After 5 days of culture, the cells were harvested and tested for cytolytic activity. Briefly, 10^4 ^{51}Cr-labeled EL4 tumor cells were mixed with varying numbers of spleen cells for 4 h and radioactivity measured in supernatants. Cultures were done in triplicate; the percentage of specific lysis was calculated as follows:

$$\% \text{ specific cytotoxicity} = \frac{\text{experimental release (counts/min)} - \text{control release (counts/min)}}{\text{maximal release (counts/min)} - \text{control release (counts/min)}}$$

where control release was that observed in wells containing 10^4 ^{51}Cr-labeled P815 target cells, and appropriate numbers of spleen cells from control cultures (see above).

Natural killer cell activity

Spleen cells (0.1 ml) from the same pool of cells used for CTL determination were incubated at various concentrations with 10^4 ^{51}Cr-labeled YAC-1 lymphoma cells (0.1 ml) as target for 4 h at 37°C in round-bottomed, 96-well plastic microtiter plates. After incubation the radioactivity in 0.1 ml of supernatant was measured in a gamma scintillation counter. Cultures were done in triplicate and the percentage of specific lysis was calculated as follows:

$$\% \text{ cytotoxicity} = \frac{\text{experimental release (counts/min)} - \text{spontaneous release (counts/min)}}{\text{maximal release (counts/min)} - \text{spontaneous release (counts/min)}}$$

where spontaneous release was obtained in the presence of thymus cells as negative effectors and maximum release by the number of counts/min present in 0.1 ml of a suspension containing 5×10^3 ^{51}Cr-labeled YAC-1 target cells.

Antibody cell-mediated cytotoxicity (ADCC)

Various numbers of spleen cells were incubated for 18 h at 37°C with 10^4 ^{51}Cr-labeled chick red blood cells (CRBC) in U well microtest plates in the presence of a 1:20,000 final dilution of rabbit anti CRBC serum. The effector to target cell ratio (E:T) varied from 100:1 to 6:1. The amount of chromium released in the supernatants was measured in a gamma counter. Cultures were done in triplicate and the percentage of specific lysis was calculated as for NK cell activity; spontaneous release was determined from the lysis in cultures containing target and effector cells but no antiserum, maximum release by the number of counts/min present in 0.1 ml of a suspension containing 5×10^3 ^{51}Cr-labeled CRBC.

Macrophage cytostatic activity

4×10^5 pooled adherent peritoneal cells from 4 mice were plated in flat bottom microplates for 2 h. After vigorous washing, 4×10^4 EL4 tumor cells/

Table 1. Lymphoproliferative response of spleen cells to T cell mitogens: effect of age and treatment with imuthiol

Mitogen	Response of 5×10^5 spleen cells		
	Untreated young BALB/c	Untreated aged BALB/c	Aged imuthiol-treated BALB/c
—	4140 ± 105	10370 ± 1426	11041 ± 611
PHA	124976 ± 2723	11233 ± 2364*	57014 ± 2940†
Con A	29008 ± 1534	11588 ± 1729*	34116 ± 2193†

*$P < 0.01$ in comparison with young untreated BALB/c.
†$P < 0.01$ in comparison with aged untreated BALB/c.

well were added to macrophage monolayers. After 18 h of incubation 1 μCi/well of (^{3}H)-thymidine was added for 6 h and radioactivity incorporated into EL4 cells was measured into a beta counter. Results are expressed in mean cpm ± S.E. of 6 cultures. Control consisted in thymidine incorporation into tumor cells in the absence of macrophages.

Evaluation of serum thymic factor (FTS)

Sixteen-month-old untreated or imuthiol-treated mice used for immunological testing were bled and serum was kept frozen until testing. The amount of FTS was measured according to the method of Bach *et al.* (1975). In brief, serum samples from 5 young mice, 8 aged untreated mice and 10 aged imuthiol-treated mice, were tested at various dilutions for their capacity to confer sensitivity to azathioprine to spontaneous rosette-forming cells.

Delayed-type hypersensitivity reaction

Six mice per group were sensitized by painting the shaved abdominal skin with a 1% solution of oxazolone (2-phenyl-4-ethoxymethylen-5-oxazolone) in acetone. The reaction was elicited 7 days later by a second application of the sensitizing agent to both sides of each ear. Changes in ear thickness was evaluated as the increment between measuring immediately before and measuring 24 h after oxazolone application using a calibrated caliper.

Graft-vs-host reaction (GVHR) mortality induced by incompatibility for H-2 antigens

(DBA/2 × C57BL/6)F1 mice (H-2^d/H-2^b) were irradiated lethally (1000 rads) using a cesium source (RX 30/55 M Irradiator, Gravatom Industries Ltd, Gosport, Hampshire, England) 24 h before grafting. Irradiated mice (8 – 10/group) received an intravenous injection of 8×10^6 spleen cells and 10^7 bone marrow cells pooled from 3 young or aged untreated or imuthiol-treated BALB/c mice (H-2^d),and mortality by GVHR was recorded.

Statistical evaluation

Student's *t*-test was used for determination of the significance of mean differences between groups.

Table 2. Lack of competitive inhibition of anti-EL4 cytotoxicity by an NK sensitive target

Cold cell type	Percentage of inhibition of cytotoxicity against ^{51}Cr EL4*									
	Young BALB/c effectors cold/hot ratio					Aged BALB/c effectors cold/hot ratio				
	5:1	10:1	20:1	40:1	80:1	5:1	10:1	20:1	40:1	80:1
BALB/c spleen cells	—	0.3	0.8	7.5	6.5	—	0	0	0	8
B6 spleen cells	—	4	16	39	46	—	0	0	32	69
EL4 cells	44	64	80	86	—	79	82	87	95	—
YAC cells	0	0	0	1	—	0	0	0	0	—

*100% of cytotoxicity against ^{51}Cr EL4 target cells by young and aged spleen cells was 30% and 3.8% respectively in this experiment for an E:T ratio of 50:1 (i.e. without cold targets).
Direct cytotoxicity against ^{51}Cr YAC target cells by young and aged spleen cells was 4.1% and 0.7% respectively for an E:T ratio of 50:1.

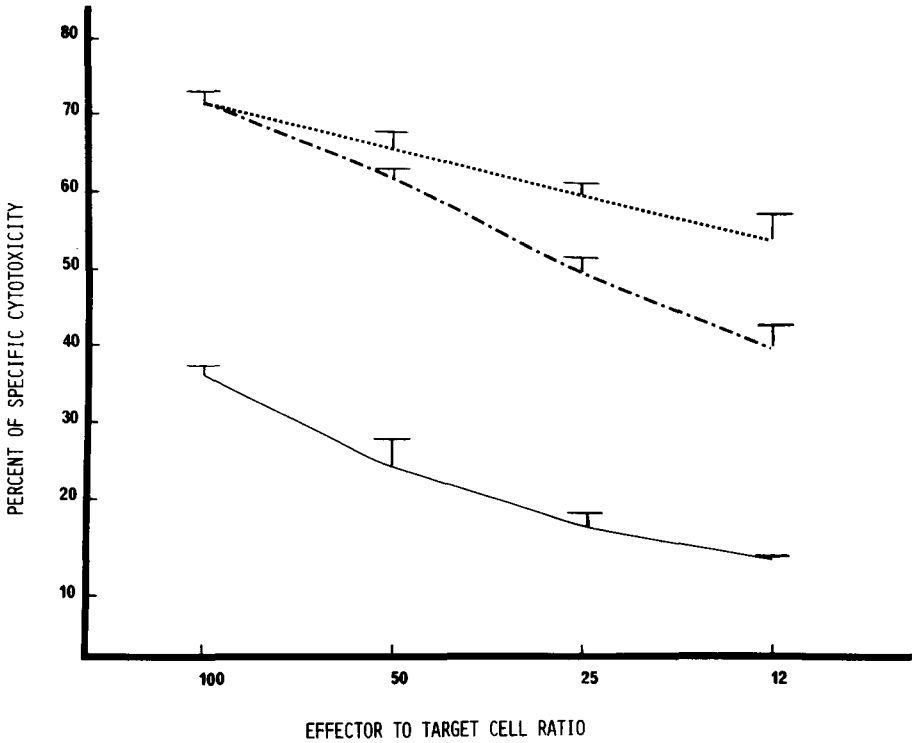

Fig. 1. Effect of age on the generation of cytotoxic T cells. Influence of imuthiol. Spleen cells from young (– – –), aged untreated (———) or aged imuthiol-treated (—·—) BALB/c mice were incubated 5 days in the presence of mitomycin-C-treated EL4 tumor cells and then tested for their capacity to lyse ^{51}Cr-labelled EL4 tumor cells at various effector to target cell ratios in a 4 h chromium release assay.

RESULTS

To evaluate the influence of repeated administration of imuthiol on the responsiveness of aged (16-month-old) female BALB/c mice, pooled spleen cells, peritoneal macrophages, and serum were sampled after 4 months of treatment for a total of 400 mg/kg imuthiol and the responses were compared to those of aged-matched and young (2–3-month-old) saline-treated animals, hereto referred as controls.

Influence of imuthiol on proliferative responses to T cell mitogens

As is shown in Table 1 (representing a typical experiment), aging was associated with inhibited responses to PHA and Con A ($P < 0.01$), inasmuch as labeled thymidine incorporation was found at similar magnitude in the presence or absence of mitogens in 16-month-old mice. A chronic treatment with imuthiol induced a 5-fold increase in the response to PHA ($P < 0.01$) and restored the response to Con A at the level of young untreated mice, and even above ($P < 0.01$).

Effect of aging on induction of cytotoxic T lymphocytes. Influence of imuthiol

Generation of cytotoxic spleen T lymphocytes (CTL) in response to foreign histocompatibility antigens bring on by EL4 (H-2^b) lymphoma cells, was the model chosen to determine whether aging interferes with a specific T cell response and whether imuthiol could restore a depressed CTL reaction. A direct NK cytotoxic activity against YAC cells is always detected in the population of sensitized lymphocytes after an MLC culture. As EL4 cells were described as NK sensitive targets (Kumar, Luevano & Bennett, 19719) we performed cold competition experiments where BALB/c spleen cells

(negative control) B6 spleen cells and EL4 cells (positive controls) and YAC cells (NK sensitive target) were added to ^{51}Cr-labeled EL4 cells. Table 2 shows that B6 and EL4 cold cell types but not BALB/c and YAC cells, absorb the cytotoxic activity against ^{51}Cr labeled EL4 developed by sensitized lymphocytes from young and aged BALB/c mice. This experiment demonstrated that the effector cells responsible for the killing of EL4 are different from those killing YAC cells.

Spleen cells from aged untreated or aged and imuthiol-treated BALB/c (H-2^d) mice were educated *in vitro* with EL4 cells and specific cytotoxicity at E/T ratios from 100:1 to 12:1 evaluated in comparison to that of spleen cells from untreated 3-month-old mice. After this incubation period, the viability of spleen cells from young, aged untreated, and aged-imuthiol-treated mice was respectively of 38, 27 and 32% of the initial number of cells at the beginning of the culture. The results of this experiment are summarized in Fig. 1. The capacity of spleen lymphocytes to differentiate into cells cytotoxic for EL4 lymphoma cells, that is to recognize foreign histocompatibility antigens, was abrogated by age in BALB/c mice. A chronic treatment with imuthiol has prevented the defective CTL response associated with aging, as the specific CTL activity of spleen cells from treated aged mice has reached the magnitude obtained with young spleen cells. The cytotoxicity against H-2^k blast cells remains closed to background level indicating the specificity of the reaction (data not shown).

Natural killer cell activity

As shown in Fig. 2, the NK cell activity against YAC-1 cells has been abolished by aging. In contrast to its efficacy in restoring the CTL activity, the chronic treatment with imuthiol was found unable to restore the NK cell activity at all the E:T ratios tested five days after the last administration.

Influence of imuthiol on the age-induced modification of antibody-dependent cell-mediated cytotoxicity (ADCC).

ADCC to chicken red blood cells (CRBC) is widely accepted as a macrophage-mediated response (Lattime, Pelus & Stutman, 1982). As illustrated on

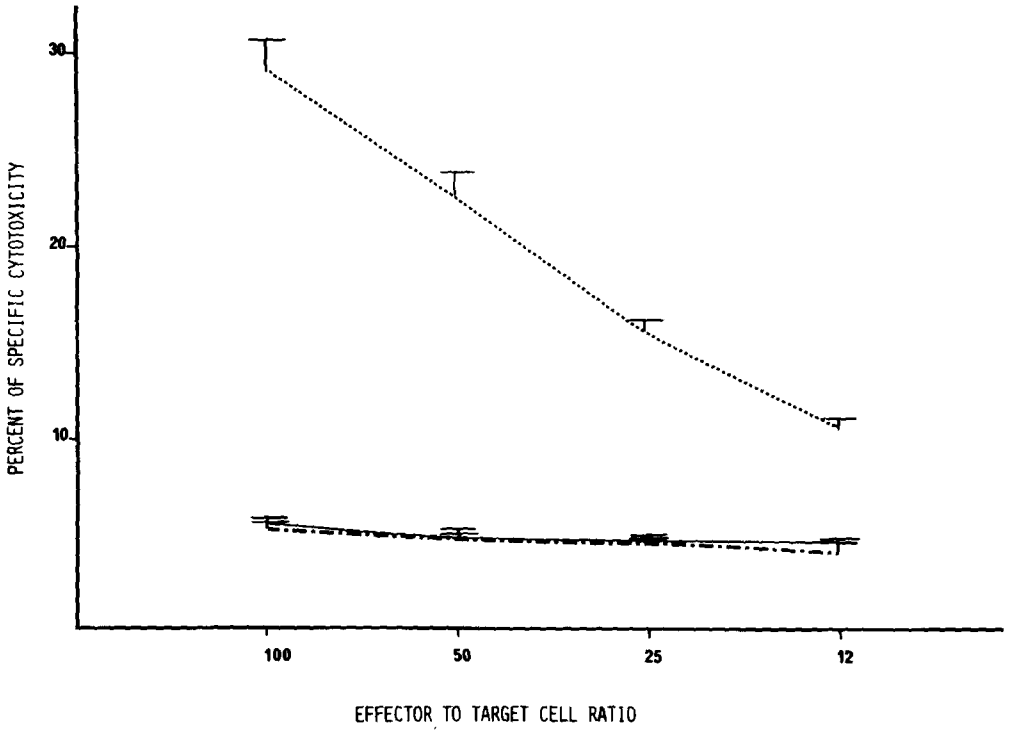

Fig. 2. Influence of imuthiol on the NK cell activity inhibited by aging. Spleen cells from young (– – –), aged untreated (———) or aged imuthiol-treated (—·—) BALB/c mice at various concentrations were tested for their capacity to kill 10^4 ^{51}Cr-labelled YAC-1 cells in a 4 h chromium release assay.

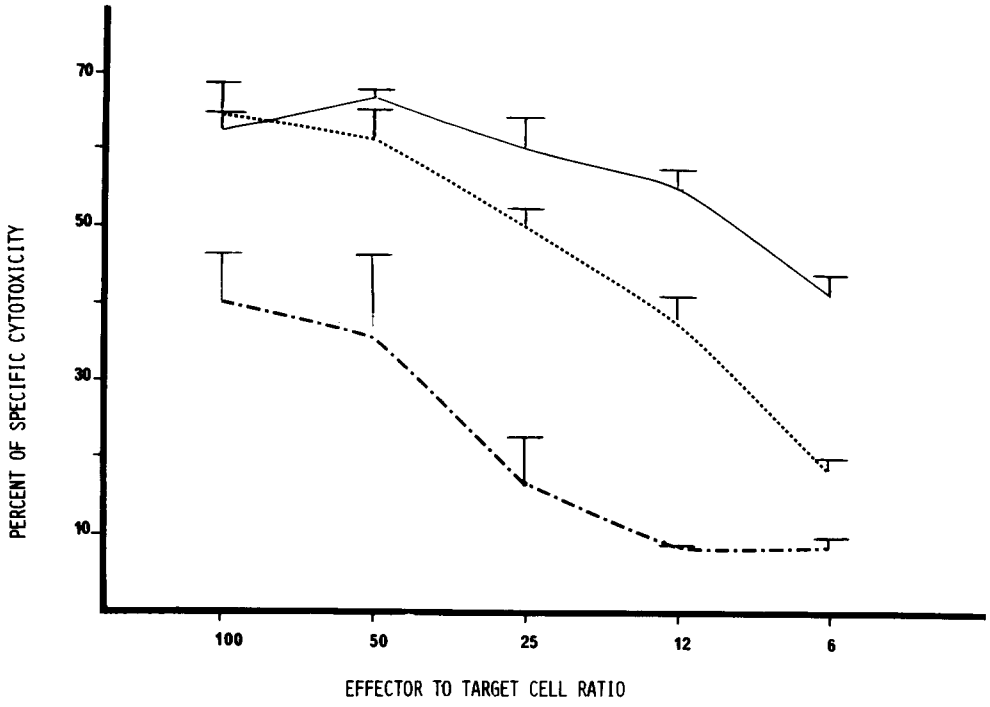

Fig. 3. Antibody-dependent cytotoxicity (ADCC) to chicken red blood cells (CRBC) of cells from aged mice. Influence of imuthiol. Spleen cells from young (– – –), aged untreated (———) or aged imuthiol-treated (—·—) BALB/c mice at various concentrations were tested for their capacity to kill 10^4 Cr-labelled CRBC in an 18 h chromium release assay.

Fig. 3, the cytotoxic activity to CRBC in the presence of anti-CRBC serum was augmented in aged mice significantly above that of young controls ($P < 0.01$) at the 25:1 or 6:1 effector to target ratios. Figure 3 shows also that a four-month treatment with imuthiol has considerably decreased the high ADCC response of aged mice far below the magnitude of response of young untreated mice.

Influence of age and imuthiol on the macrophage cytostatic activity for tumor cells

The effect of aging on peritoneal macrophage cytostasis for tumor cells was evaluated against EL4

Table 3. Macrophage cytostatic activity in BALB/c mice. Effect of age and treatment with imuthiol

Thymidine incorporation (mean counts/min ± S.D.) into 4×10^4 EL4 tumor cells in the presence of 4×10^5 macrophages from	
Untreated 2–3-month-old BALB/c mice	5533 ± 1452*
Untreated 14–15-month-old BALB/c mice	16509 ± 3230
Imuthiol-treated 14–15-month-old BALB/c mice	74296 ± 1877*
No macrophages (control group)	25000 ± 8401

*$P < 0.01$ in comparison with the control group.

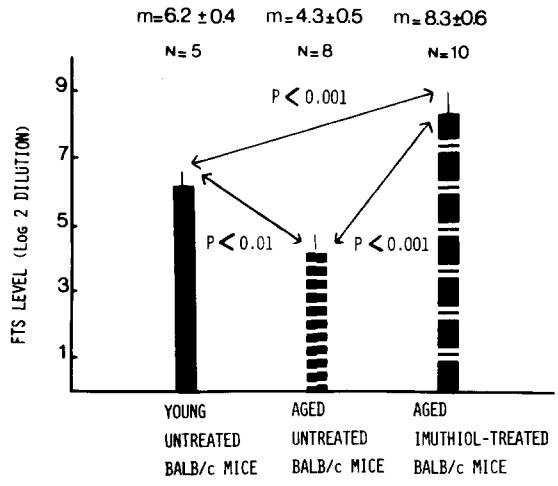

Fig. 4. Facteur thymique sérique (FTS). Influences of aging and treatment with imuthiol. Mean FTS levels ± S.D. (expressed in log 2 dilution) in serum of individual BALB/c mice. (N = number of animals and M = means FTS levels).

lymphoma cells, which tumor cells were already employed to test for cytotoxic T lymphocyte generation (Fig. 1).

The results summarized in Table 3 show that aging caused a significant loss in macrophage cytostatic activity ($P < 0.01$) against EL4 cells compared with this activity in young BALB/c mice. Indeed, the proliferation of EL4 cells, measured in terms of labeled thymidine incorporation in the presence of "aged" macrophages, was found at a level not differing from that of EL4 cells cultured in the absence of macrophages (NS). The treatment with imuthiol of aged mice before macrophage sampling has resulted in a significant increase in EL4 cell proliferation ($P < 0.01$) beyond that of aged controls. The decreased ADCC and cytostatic activity mediated by macrophages demonstrated an inhibiting action of imuthiol on macrophage functions.

Evaluation of FTS in serum

Means of FTS titers, expressed as the $\log_2$ of the active serum dilution tested individually in mice from each group, are presented in Fig. 4. Aging induced a loss in FTS activity as the amount of FTS in the serum of the 8 aged controls ($m = 4.3 \pm 0.5$) was significantly lower than the activity of the serum of 5 young mice ($m = 6.2 + 0.4$) ($P < 0.01$). Repeated administration of imuthiol to aged mice leads to a significant increment of FTS in the serum of 10 treated mice ($m = 8.3 + 0.6$, $P < 0.001$). This amount of FTS activity was found also significantly higher ($P < 0.01$) than that observed in the serum of untreated young BALB/c mice.

In vivo *testing of influence of imuthiol on immunological senescence*

The influence of repeated administration of imuthiol on the immune responsiveness of aged female BALB/c mice was assessed by *in vivo* testing of delayed-type hypersensitivity (DTH) to oxazolone, a response involving macrophages and T cells, and by a graft-vs-host (GVH) reaction, as a model of T – T cell interaction.

Influence of imuthiol of age-induced impairment of delayed-type hypersensitivity (DTH)

The effect of imuthiol was studied by comparing the DTH reactivity on oxazolone of young mice and old mice (6 mice per group). As depicted in Fig. 5, aging has lowered the DTH response in aged BALB/c mice ($m = 2.3 \pm 1.0$) significantly ($p = 0.05$) to that of young mice ($m = 3.8 \pm 1.3$). The efficiency of imuthiol in restoring *in vivo* the T cell and macrophage responses evaluated in terms of DTH to oxazolone and impaired by aging is illustrated in Fig.

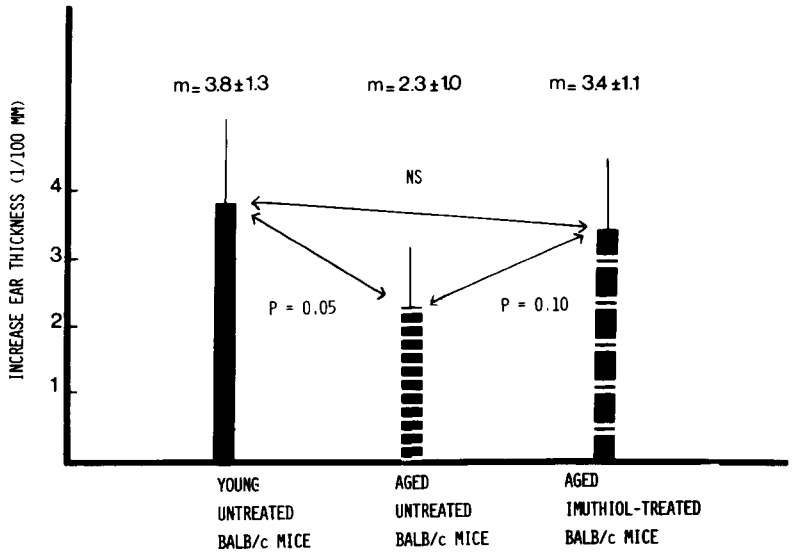

Fig. 5. Delayed-type hypersensitivity to oxazolone. Influences of aging and treatment with imuthiol. (M = mean increase ear thickness ± S.D. of six mice per group).

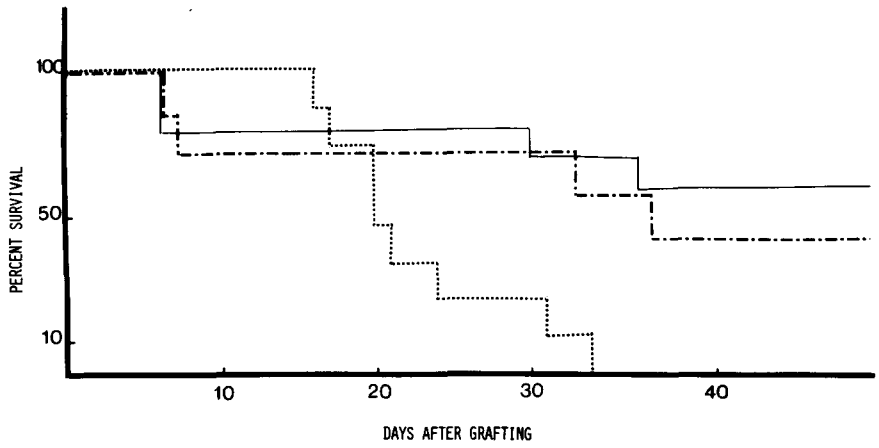

Fig. 6. Mortality following graft-vs-host reaction (GVHR) induced by incompatibility for H-2 antigens. Influences of aging and treatment with imuthiol. Bone marrow, and spleen cells from young (– – –), aged untreated (———), or aged imuthiol-treated (—·—) BALB/c mice were grafted i.v. to lethally irradiated (C57BL/6 × DBA/2)F1 mice.

5. The DTH response was indeed increased (m = 3.4 ± 1.1) yet did not significantly differed from that of young controls (P = 0.10), although it did not also differed from the mean DTH magnitude of aged animals (P = 0.1). In previous experiments, non-specific DTH reactions were not observed when picryl chloride was used as sensitizing agent.

Graft-vs-host (GVH) mortality by incompatible H-2 antigens

Spleen and bone marrow cells from either young untreated, aged untreated or aged and treated female BALB/c mice (H-2^d) were injected via the intravenous route to lethally irradiated (C57BL/6 × DBA/2) F1 female mice (H-2^b/H-2^d). As is shown in Fig. 6, all hybrid mice grafted with young BALB/c cells died with a median survival time of 21 days. In contrast, 60% of the recipient mice survived to grafting with cells of aged and untreated BALB/c mice. A 4-month chronic treatment with imuthiol did not significantly modify the mortality rate induced by cells of untreated aged animals, and 43% mice remained alive throughout the whole period of observation.

DISCUSSION

In view of its efficiency in regulating T cell-dependent activities in young animals, it was considered important to determine whether imuthiol would also correct or restore the age-dependent decline in T cell potential. The age of 16 months was selected to evaluate the effect of a chronic treatment on immunodepression associated with age since in BALB/c mice most of the immune reactions are low at that age and since the median life span is about 18 months in this strain of mice (Walters & Claman, 1975).

In this context, a long-term treatment with imuthiol can correct the T-cell impaired function associated with aging in BALB/c mice: the capacity of T cells to proliferate upon mitogen stimulation or to differentiate into cytotoxic T cells (CTL) after allogeneic stimulation was increased, and even in some cases restored at the magnitude of young controls, in aged mice treated for 4 months with imuthiol.

Although the direct NK activity of spleen cell suspension of aged and imuthiol-treated mice was negative before any culture (Fig. 1), the release of interleukin 2 (IL-2) and interferon (IFN) during the mixed lymphocyte reaction may have enhanced NK activity. Part of the T cell cytotoxicity can be achieved by NK cells since EL4 cells have been described as an NK sensitive target (Kumar, Luevano & Bennett, 1979). However, results of the cold target inhibition experiments clearly show that EL4 cytotoxicity is blocked by B6 normal cells but not by YAC cells, indicating that the cytotoxicity is mainly achieved by T cells. These findings confirm that imuthiol displays its effects on T-cell associated events (Renoux, 1982), and even after repeated administration (Renoux & Renoux, 1981b), which effects contrast with the dose- and time-inhibition

induced by most immunomodulators. Concurrently, imuthiol increases the thymus hormone-like (FTS) activity in aged mice above the level of young animals.

Previous reports have shown that imuthiol enhances the release by liver cells (Renoux & Renoux, 1981a) of a factor specifically active on the T cell lineage and even on *nu/nu* mice (Renoux & Renoux, 1983; Renoux, Touraine & Renoux, 1980) and concomitantly induces cell proliferation in the T cell rich areas of lymphoid organs (Pompidou, Renoux, Guillaumin, Mace, Michel, Coutance & Renoux, 1984). It is of interest that a test such as cell sensitivity to azathioprine, initially applied to the determination of FTS (Bach, Bach, Blenot, Bucas, Charriere, Dardenne, Fournier & Pleau, 1978) can also be useful to titrate the imuthiol-induced serum factor. The imuthiol-induced serum factor was demonstrated in young mice as well as in nude mice to stimulate precursor cells to differentiate into T cells, then trigger the different steps of T-cell maturation (Renoux & Renoux, 1980, 1981a). This increase in T-cell specific differentiating factor might account for the mechanism of action of imuthiol in aged animals. FTS was indeed demonstrated to allow the generation of IL-2 producer cells in the nude mouse (Palacios, Fernandez & Sideras, 1982). Moreover, recent work in our laboratory indicates that imuthiol treatment corrects the deficient IL-2 production by helper T cells of aged BALB/c mice (personal communication). These data strongly suggest that imuthiol induce the production of a factor displaying FTS activity which in turn trigger the activation of deficient T cells into IL-2 producing helper cells leading to a restoration of lectin-initiated proliferation and cytotoxic T cell-differentiation. However, it is not known whether imuthiol acts on the remanant of thymus epithelial cells or on liver cells for the production of the FTS-like activity found in the serum of aged treated mice.

The above results suggest that imuthiol acts on various T-cell subsets including helper and cytotoxic cells. However, the agent's action on the interacting and complex immune system probably involves an intricated pathway, in view of the other assays. The DTH response was moderately increased in treated mice and the impaired GVH reaction in a H-2 incompatible system was not modified by treating aged donor mice. A synergistic T – T cell interaction is involved in lethal GVH reactions and the expression of different effector functions may be initiated by distinct cell subsets (Tigelaar & Asofsky, 1972). Current studies attempt to determine whether imuthiol would act on a T-cell subset essential in graft rejection or modify the expression of cell-surface histocompatibility antigens, so that donor cells from treated animals are rendered unable to recognize non-self H-2 antigens, although these cells possess increased T-cell reactivity *in vitro*. DTH reaction involved monocyte – lymphocyte interactions and that only a trend in favor of an increased response was observed in aged treated mice may be due to a macrophage defective function as shown by impaired tumor cell lysis, which function cannot be overcome by the stimulating influence of the agent on T cells.

Imuthiol is unable in the present assays to modify the NK activity depressed by aging. Previous findings indicate that imuthiol activates or suppresses NK cell activity at time depending on the mouse age and time of administration (Renoux, Bardos, Degenne & Musset, 1982). The low NK activity of aged mice does not exclude the possibility that under a stimulation, NK cell responses would be greater in the imuthiol treated group. Indeed Weindruch, Devens, Raff & Walford (1983) demonstrate that the influence of a caloric restriction on the age-related decline in NK activity was apparent only after Poly I-C administration. Alternatively, the finding might be interpreted in the light of reports indicating a balance between the extent of NK response and cytotoxic T cell activity (Vanky & Argov, 1980) as it could be seen in the present study where the treatment was found to increase the CTL response.

While the basis of target recognition and kill in ADCC to CRBC appear to be quite distinct from those in macrophage cytostatism (Adamo & Johnson, 1982; Lattime, Pelus & Stutman, 1982), the two effector mechanisms are mediated by macrophages. Imuthiol inhibits the macrophage-induced tumor killing, yet brings near to the level of young controls the magnitude of ADCC to CRBC of aged mice. The nature of the change in ADCC to CRBC is unclear as imuthiol was found unable to modify the antibody-dependent cytotoxicity response in young mice (Bardos, Degenne, Lebranchu, Bizière & Renoux, 1981). The inhibited cytotoxic influence against tumor cells of macrophages from untreated aged mice might be attributable to a lack of superoxide anion release in medium, one of the mechanisms involved in tumor killing by macrophages (Oyanagui, 1982). Current studies indicate indeed that imuthiol can depress the production of superoxide dismutases and increase gluthatione reductase level in macrophages, thus modifying the oxygen intracellular pathway and liberating non-toxic oxygen products in the

surrounding. However, imuthiol can activate some macrophage activities in young mice such as colloidal gold clearance or intracellular listericidal activity but does not induce hypertrophy or hyperplasia of the reticulo-endothelial system (Renoux, 1982; Neveu & Vincendeau, 1983). The influence of imuthiol on mouse life span has not been evaluated, as it has already been shown that treatments with either 17 or 140 mg/kg daily for at least 100 consecutive weeks induce a prolonged survival of female mice and a reduced incidence of spontaneous-arising tumors (National Cancer Institute, 1979).

It is obvious from the above that the chronic administration of imuthiol restores the T-cell function inhibited by aging and likely through regulatory processes where FTS may play a role. The beneficial influence of imuthiol on the T-cell lineage is not associated with toxic effects. The model of aging mice allows to select compounds exhibiting immunorestorative capacity whose stimulating activity may not be detectable in the normal immune state. Consequently substances detected using this model may be expected to have therapeutic value in other circumstances where immunodeficiencies occur.

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