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AGING AND ELICITING AGENTS: EFFECT ON MURINE PERITONEAL MACROPHAGE MONOKINE BIOACTIVITY

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Abstract — Decreased responsiveness of the aged to infection may be associated with a decline in monokine production. Prior studies in macrophages have used different eliciting agents, and results have varied. We assessed the effect of age on interleukin-1 (IL-1), tumor necrosis factor (TNF), and interleukin-6 (IL-6) in unelicited, thioglycollate (TG)-elicited, and complete Freund's adjuvant (CFA)–elicited peritoneal macrophages. Resident macrophages or CFA-elicited macrophages from middle aged or aged mice produced significantly less monokine bioactivity than resident or CFA-elicited macrophages from young mice. Monokine bioactivity from TG-elicited macrophages from aged and middle aged mice was significantly increased when compared with macrophages of young mice. Eliciting agents may alter macrophage populations and interactions with other cells leading to changes in monokine bioactivity with aging.

Key Words: interleukin-1, interleukin-6, tumor necrosis factor, complete Freund's adjuvant, thioglycollate, age

INTRODUCTION

THE DIMINISHED response of the aged host to infection has been well described. A decline in the febrile response, trace metal responses, and diminished clearance of organisms with increasing age has been noted. In particular, the aged have difficulty in containing intracellular pathogens (Gardner and Remington, 1977; Emmerling *et al.*, 1979; Louria *et al.*, 1982). The monocyte–macrophage system plays a critical role in the acute phase response and containment of pathogens. Despite the inability of the aged host to respond to infection, few clear-cut defects in in-vitro monocyte–macrophage function have been found (Perkins, 1971; Johnson *et al.*, 1978; Gardner *et al.*, 1981; Caperna and Garvey, 1982; Finger *et al.*, 1982; Wustrow *et al.*, 1982; Antonaci *et al.*, 1984; Petrequin and Johnson, 1984; Esposito *et al.*, 1988; Lavie and Gershon, 1988; Chen *et al.*, 1991). A diminished ability to produce the monokines/endogenous pyrogens, IL-1 and TNF, in the aged has

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been described by some investigators using febrile response assays (Norman *et al.*, 1988), bioactivity assays (Bruley-Rosset and Vergnon, 1984; Hayari *et al.*, 1984; Inamizu *et al.*, 1985; Bessler *et al.*, 1989; Bradley *et al.*, 1989; Rudd and Banerjee, 1989; Davila *et al.*, 1990) and enzyme-linked immunosorbent assay (ELISA) methods (Mooradian *et al.*, 1991); however, others have not found these defects (Rosenberg *et al.*, 1983; Jones *et al.*, 1984; Kauffman, 1986; Amadori *et al.*, 1988; Bradley *et al.*, 1990; Putnam and Peterson, 1991).

This variation in experiments assessing monokine production by the aged might relate to differences in technique, especially methods to obtain macrophages. Different agents used to elicit peritoneal macrophages for study have been shown to alter macrophage function (Edelson *et al.*, 1975; Johnston *et al.*, 1978; North, 1978; Ruco and Meltzer, 1978; Hopper and Geczy, 1980; Ogmundsdottir, 1980; Spitalny, 1981; Rasmussen *et al.*, 1983; Leijh *et al.*, 1984; Blanckmeister and Sussdorf, 1985; Hopper, 1986; Tannenbaum *et al.*, 1987; Melnicoff *et al.*, 1989; Higuchi *et al.*, 1990; Reed and Burnham, 1991; Tomioka and Saito, 1992) and possibly monokine production (Marcinkiewicz, 1991) through mechanisms that are not yet well defined. In this study, the effects of various eliciting agents, complete Freund's adjuvant (CFA), thioglycollate (TG), or no treatment, on IL-1, IL-6, and TNF bioactivity in peritoneal macrophage supernatants from young, middle aged, and aged mice was assessed.

MATERIALS AND METHODS

Mice

Young (2–3 months), middle aged (12–13 months), and aged (23–25 months) C57BL/6 male mice were obtained from the University of Michigan Core Facility for Aged Rodents or from the National Institute on Aging. Animals were housed at 26°C, and given chow and water ad libitum. Macrophages were not used in the study if the donor mice appeared ill or gross anatomic abnormalities were noted at the time of harvest of peritoneal exudate cells.

Macrophage isolation and culture

Mice in each age group were injected intraperitoneally with 3 ml of 3% TG (Difco, Detroit), 0.5 ml CFA (Difco) that had been emulsified 1:1 in pyrogen-free saline, or received no injection of an eliciting agent (resident macrophages). Peritoneal exudate cells were harvested 4 days following injection of TG and 14 days following injection of CFA by washing the abdominal cavity with 6 ml Hanks' balanced salt solution (HBSS) (JRH Biologicals, Lenexa, KS).

The cells were washed, counted, and the percentage of cells which were macrophages was assessed morphologically and with nonspecific esterase stain (Sigma, St. Louis). A final concentration of 2×10^6 cells/ml was added to RPMI 1640 medium containing 2 mM L-glutamine, 5 u/ml penicillin, and 5 μg/ml streptomycin (JRH Biologicals). One ml of cell suspension was added to each well of a 24-well flat-bottomed plastic plate (Flow Laboratories, McLean, VA), and plates were incubated at 37°C in 5% CO_2.

After 1 h, the nonadherent cells were removed by two vigorous washings with HBSS. For each mouse, adherent cells from one well were removed with a rubber policeman and

counted. The adherent cells in the remaining wells were incubated in the above medium with 10 μg/ml *Escherichia coli* 0111:B4 lipopolysaccharide (LPS) (Difco). Supernatants were collected from different wells at 1 or 2, 4, 8, 24, and 48 h following LPS stimulation, centrifuged to remove cellular debris, and stored at −70°C prior to assay for monokines.

Monokine bioassays

TNF-α concentrations in the supernatants were determined by a modified cytotoxicity assay using the WEHI 164 subclone 13 murine fibrosarcoma cell line (gift of Dr. Steven Kunkel) (Espevik and Nissen-Meyer, 1986). Serial 1:2 dilutions of supernatants or human recombinant TNFα (rhTNF) Genzyme, Boston, MA) were made in complete medium containing RPMI-1640, 2 mM L-glutamine, 5 u/ml penicillin, 5 μg/ml streptomycin, and 10% heat-inactivated mycoplasma-free fetal calf serum (JRH Biologicals). A dilution of supernatant or standard was added in triplicate in 100-μl aliquots to each well of a 96-well flat bottom culture plate (Flow Laboratories). WEHI cells were adjusted to a concentration of 5×10^5 cells/ml in complete media, and 100-μl aliquots were added to the wells which contained either supernatants or standards. The plates were incubated for 18 h at 37°C in 5% CO_2.

IL-1 concentrations in the supernatants were determined by a proliferation assay using the D10.G4.1 T helper cell line (ATCC #TIB224, Rockville, MD) (Kaye *et al.*, 1983). Serial 1:3 dilutions of supernatants or human concensus IL-1 (hIL-1) (Endogen, Boston) were made in complete medium containing Click's medium (Irvine Scientific, Santa Ana, CA), 2.5 μg/ml concanavalin A (Pharmacia, Piscataway, NJ), 10% fetal calf serum, 2 mM 2-mercaptoethanol, 2 mM L-glutamine, 5 u/ml penicillin, and 5 μg/ml streptomycin. One-hundred-microliter aliquots of each supernatant or standard dilution were added in triplicate to each well of a 96-well flat-bottomed plate. D10.G4.1 cells were adjusted to a concentration of 2×10^5 cells/ml in complete media, and 100-μl aliquots were added to the wells which contained either the supernatants or standards. The plates were incubated for 48 h at 37°C in 5% CO_2.

IL-6 concentrations in the supernatants were determined by a proliferation assay using the IL-6-dependent B9 hybridoma cell line (gift of Drs. L.A. Aarden and M.J. Kluger) (Helle *et al.*, 1988). Serial 1:3 dilutions of supernatants or human recombinant IL-6 (rhIL-6) (Genzyme, Boston, MA) were made in 0.2 ml of Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY) containing 10% fetal calf serum, 2 mM 2-mercaptoethanol, 5 u/ml pencillin, and 5 μg/ml streptomycin. One-hundred-microliter aliquots of each supernatant or standard dilution were added in triplicate to each well of a 96-well flat-bottomed plate. The B9 cells were adjusted to 5×10^4 cells/ml in complete media, and 100-μl aliquots were added in triplicate to each well containing either supernatants or rhIL-6. The cells were incubated for 72 h at 37°C in 5% CO_2.

^{3}H-thymidine incorporation

Monokine proliferative and cytotoxic activities were measured by ^{3}H-thymidine incorporation. ^{3}H-thymidine (ICN, Costa Mesa, CA) (1 μCi) was added to all wells. IL-1 and TNF assay plates were incubated for 8 h and IL-6 assay plates were incubated for 4 h at 37°C in 5% CO_2. The cells were harvested and thymidine incorporation was measured in a beta counter.

Calculation of monokine concentrations

Standard curves were drawn by comparing dpm (y axis) versus the log of known concentrations of rhIL-1 or rhTNF (x axis) for each experiment. The linear equation that described the straight portion of the recombinant standard curve was obtained using a computer graphics program (Cricket Software, Malvern, PA). The dpm measured for each supernatant dilution (y) was used in the equation to calculate the concentration of murine monokine (x) present in the dilute supernatant. The limits of detection were 15 pg/ml for TNF and 0.1 pg/ml for IL-1 in our assays.

To assess IL-6 activity, a separate curve was drawn for each mouse (dpm [y] vs. supernatant dilution [x]). One half of the maximum proliferative response (defined as 1 unit of IL-6 activity) was calculated from each curve for each mouse. An equation ($y = mx + b$) was derived by linear regression analysis to define the straight portion of each curve. The dpm for each mouse half-maximal response (y) was used in each equation to calculate the dilution of supernatant (x) that contained 1 unit of IL-6 activity for each mouse. The reciprocal of that dilution represented the number of units of IL-6 in the undiluted supernatant.

A standard curve for rhIL-6 was also drawn (dpm vs. concentration [pg/ml]) for each experiment. The concentration of rhIL-6 resulting in one-half maximal proliferation was calculated. A unit of IL-6 activity for each mouse could then be compared against a unit of rhIL-6 activity of known concentration. By converting the murine IL-6 units to equivalent units of rhIL-6, a final concentration in pg/ml could be calculated for each murine supernatant. We found that 1 u/ml of murine IL-6 activity in our assay generally corresponded to 2 u/ml or 200 pg/ml of rIL-6. The limit of detection of rhIL-6 was 50 pg/ml in our assay.

Assay controls

Proliferation or cytotoxicity was assessed using complete medium alone or cell lines in complete medium as a control to assure that nonspecific activation had not occurred. In addition, in each experiment, triplicate wells were set up with supernatants from macrophages which had not been stimulated with LPS. Antibodies for each murine monokine (Genzyme) were used in their respective assays to ascertain that the proliferative or cytotoxic response was specific for that monokine. Polyclonal rabbit antimurine TNFα (1:10) was added to 5 different murine supernatants, polyclonal rabbit antimurine IL-1 (1:10) was added to supernatants from 2 different mice, and monoclonal rat antimurine IL-6 (1:50) was added to supernatants from 3 different mice, each time using the appropriate specific cell line.

Monokine ELISA methods

CFA-elicited macrophage supernatants, from young, middle aged, and aged mice, were tested by ELISA for the three monokines. Supernatants from macrophages that had been incubated with LPS for 24 h were selected for TNF measurement, 8-h supernatants were used for IL-1 measurement, and 4-h supernatants for IL-6 measurement. Undiluted supernatants and supernatants diluted severalfold in RPMI were assayed in duplicate in an ELISA for murine TNFα (Genzyme, Boston), IL-1α, and IL-6 (Endogen, Boston). Limits of detection were 50 pg/ml for TNFα and 15 pg/ml for IL-1α and IL-6.

Statistical analysis

The effect of age on the bioactivity of each monokine and cell yields was determined by one-way analysis of variance.

RESULTS

Monokine bioactivity in CFA-elicited macrophages

While TNF bioactivity was detectable at 2 h (data not shown), the highest levels were found at 4 h, with a rapid decline thereafter in supernatants from all age groups (Fig. 1A). Supernatants from aged and middle aged mice consistently contained less TNF than supernatants from young mice at all time-points; these results were significant at 8, 24, and 48 h.

For IL-1 in all age groups, supernatant bioactivity was barely detectable at 1 h (data not known), and readily measured at 4 and 8 h, with the peak response at 24 h, followed by a decline at 48 h (Figure 1B). Supernatants from aged and middle aged mice showed significantly less IL-1 activity at 8 and 24 h than supernatants from young mice.

IL-6 activity was detectable at 1 h (data not shown), and showed only modest rises through 48 h. Supernatants from aged mice contained significantly less IL-6 activity than supernatants from middle aged and young mice at 4 h (Fig. 1C). After that, no significant age-related differences were apparent.

Monokine bioactivity in TG-elicited macrophages

Major differences were apparent when monokine bioactivity was assessed in supernatants from TG-elicited macrophages. Increasing age did not decrease monokine bioactivity, as seen with CFA-elicited macrophages. In fact, at many time-points supernatants from TG-elicited aging macrophages showed more monokine bioactivity than those from young mice.

Although TNF bioactivity was detectable at 2 h (data not shown), peak TNF levels were not found at 4 h (as noted with CFA-elicited cells), but at 8 and 24 h (Fig. 2A). In contrast to CFA results, supernatants from TG-elicited macrophages from aged and middle aged mice contained more activity than supernatants from young mice at 4, 8, and 24 h.

In TG-elicited macrophage supernatants, IL-1 activity was very low when compared to levels noted in supernatants from macrophages elicited with CFA (note change in scale of the graph), and peak levels were found at 48 h (Fig. 2B). Macrophages of aged mice produced more IL-1 than macrophages of young and middle aged mice, with a significant effect of increasing age noted at all time-points.

IL-6 bioactivity was significantly greater than TNF and IL-1 in TG-elicited macrophage supernatants, and greater than that noted in CFA-elicited macrophages. IL-6 was detectable at 2 h (data not shown), and peak IL-6 levels were observed at 24 and 48 h (Fig. 2C). At 8, 24, and 48 h, supernatants of macrophages from aged and middle aged mice contained more IL-6 than supernatants from young mice, but a statistically significant effect of age was found only at 8 h.

Monokine bioactivity in resident macrophages

In general, results with resident peritoneal macrophages mirrored those found with CFA-elicited macrophages. However, far less TNF was produced by the resident macrophages than those elicited with CFA or TG (note difference in scale of the graph) (Fig. 3A).

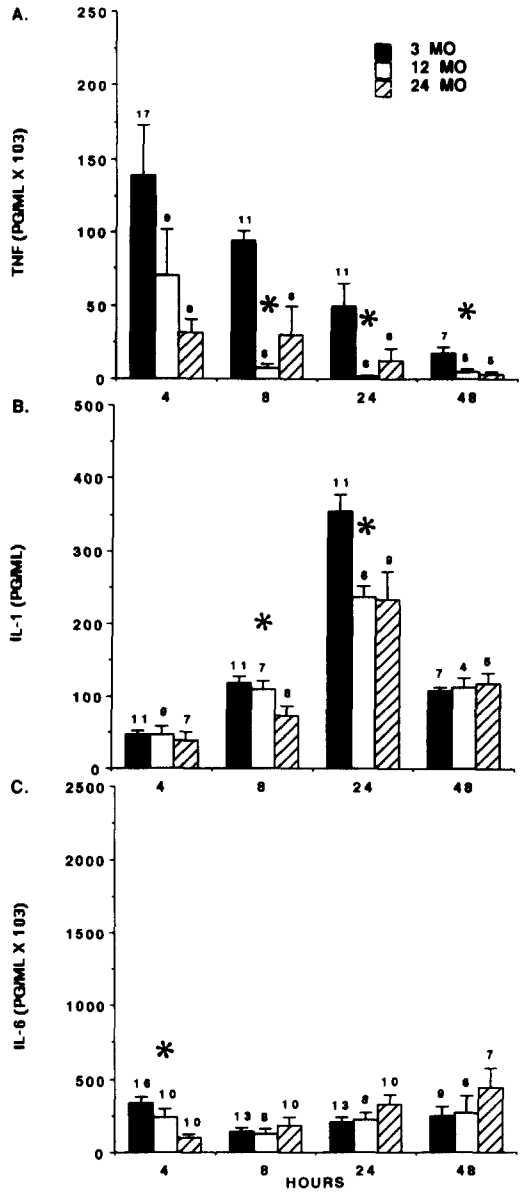

FIG. 1. Monokine bioactivity in supernatants from CFA-elicited peritoneal macrophages from three age groups of mice stimulated with LPS in vitro. All data are expressed as mean ± SE. Statistical differences were assessed by one-way analysis of variance. * = significant time-point. (A) TNF: Supernatants from aged and middle aged macrophages contained significantly less TNF activity than supernatants from young macrophages at 8 h ($p = 0.0001$), 24 h ($p = 0.028$), and 48 h ($p = 0.013$). (B) IL-1: Supernatants from aged macrophages contained significantly less IL-1 activity than supernatants from middle aged and young macrophages at 8 h ($p = 0.023$) and 24 h ($p = 0.008$). (C) IL-6: Supernatants from aged and middle-aged macrophages contained significantly less IL-6 activity than supernatants from young macrophages at 4 h ($p = 0.002$).

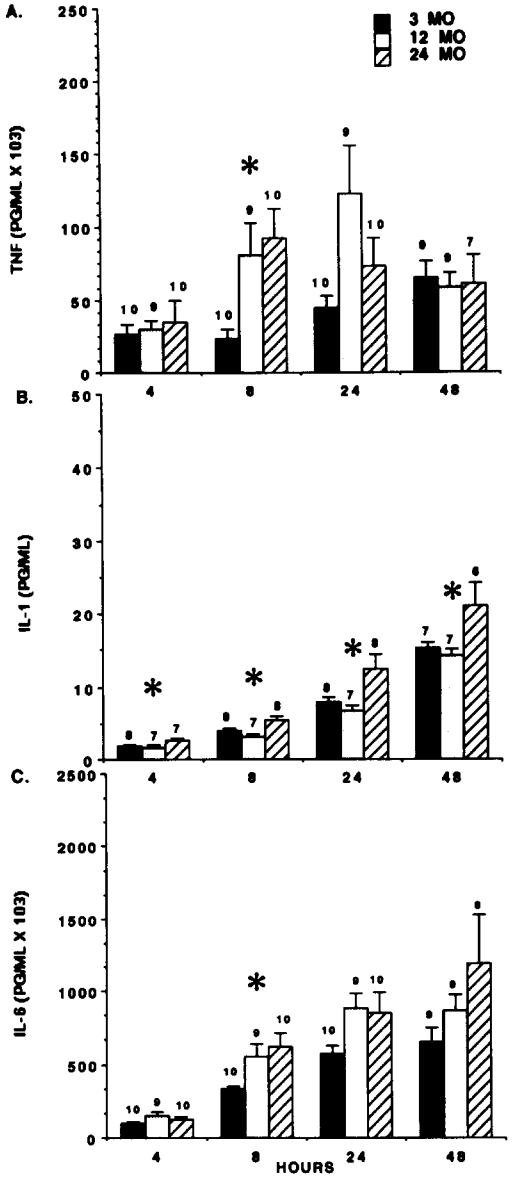

FIG. 2. Monokine bioactivity in supernatants from TG-elicited peritoneal macrophages from three age groups of mice stimulated with LPS in vitro. All data are expressed as the mean ± SE. Statistical differences were assessed by one-way analysis of variance. * = significant time-point. (A) TNF: Supernatants from aged and middle aged macrophages contained significantly more TNF activity than supernatants from young macrophages at 8 h ($p = 0.033$). (B) IL-1: Supernatants from aged macrophages contained significantly more IL-1 activity than supernatants from middle aged and young macrophages at 4 h ($p = 0.05$), 8 h ($p = 0.005$), 24 h ($p = 0.011$), and 48 h ($p = 0.039$). (C) IL-6: Supernatants from aged and middle aged macrophages contained significantly more IL-6 activity than supernatants from young macrophages at 8 h ($p = 0.02$).

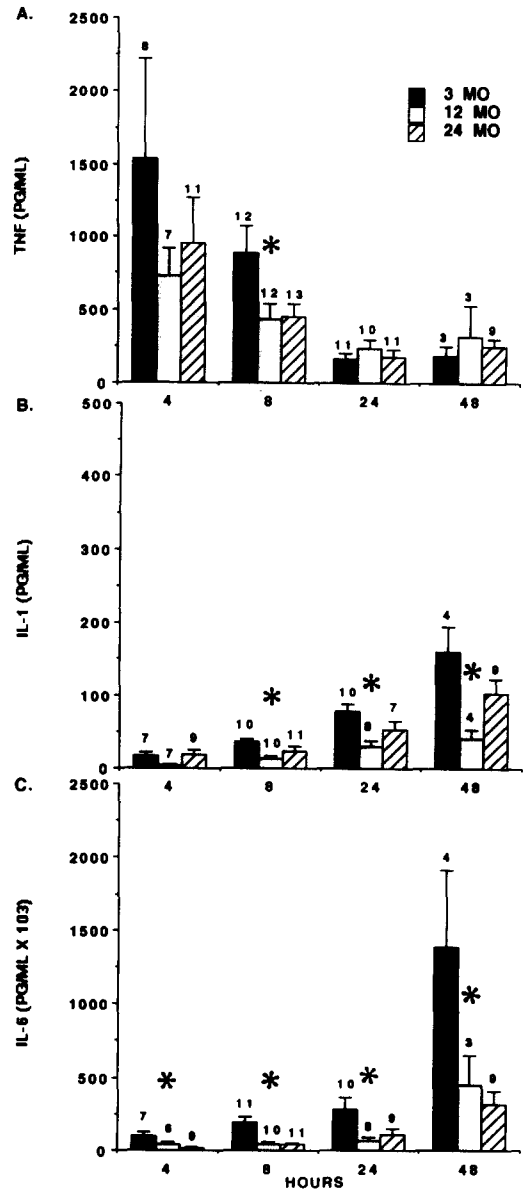

FIG. 3. Monokine bioactivity in supernatants from resident peritoneal macrophages from three age groups of mice stimulated with LPS in vitro. All data are expressed as the mean ± SE. Statistical differences were assessed by one-way analysis of variance. * = significant time-point. (A) TNF: Supernatants from aged and middle aged macrophages contained significantly less activity than supernatants from young macrophages at 8 h ($p = 0.043$). (B) IL-1: Supernatants from aged and middle aged macrophages contained significantly less activity than supernatants from young macrophages at 8 h ($p = 0.007$), 24 h ($p = 0.004$), and 48 h ($p = 0.031$). (C) IL-6: Supernatants from aged and middle aged macrophages contained significantly less activity than supernatants from young macrophages at 4 h ($p = 0.008$), 8 h ($p = 0.0001$), 24 h ($p = 0.035$), and 48 ($p = 0.024$).

As noted with CFA-elicited macrophages, peak TNF levels were detected at 4 h. Supernatants from resident macrophages of aged and middle aged mice contained less TNF than supernatants from macrophages of young mice, but significant differences were seen only at 8 h.

IL-1 bioactivity in supernatants from resident macrophages was almost as great as that seen in CFA-elicited macrophages, and much greater than that seen in thioglycollate-elicited macrophages (Fig. 3B). IL-1 levels peaked at the same time as IL-1 levels from TG-elicited macrophages (48 h) and later than IL-1 levels from CFA-elicited macrophages (24 h). Supernatants from resident macrophages of aged and middle aged mice contained less IL-1 than supernatants from macrophages of young mice, and a significant effect of age was found at 8, 24, and 48 h.

Levels of IL-6 detected in resident macrophage supernatants were similar to levels detected in CFA-elicited and TG-elicited supernatants. Peak IL-6 bioactivity was detected at 24 h, as noted in TG-elicited macrophages (Fig. 3C). Supernatants from resident macrophages of aged and middle aged mice contained significantly less IL-6 bioactivity when compared with supernatants from macrophages of young mice at 4, 8, 24, and 48 h.

Assay controls

Supernatants from macrophages cultured in medium without LPS produced no measureable monokines. When ^{3}H-thymidine was added to complete medium alone, or WEHI, D10.G4.1 cells, and B9 cells in their respective complete media, only minimal cytoxicity (WEHI cells) or proliferation (D10.G4.1 and B9 cells) was noted, showing no nonspecific activation by medium or cells alone (Fig. 4). Addition of rhTNF to WEHI cells resulted in a marked decline in ^{3}H-thymidine incorporation, showing increased cytotoxicity. In contrast, addition of hIL-1 to D10.G4.1 cells or rhIL-6 to B9 cells resulted in marked incorporation of ^{3}H-thymidine, reflecting increased proliferation. The murine supernatants tested in these experiments showed activity similar to the standards in all three assays.

Addition of antimurine TNFα to murine supernatants abolished cytotoxic activity in the WEHI assay by 85%. Addition of antimurine IL-1 to murine supernatants abolished proliferative activity in the D10.G4.1 assay by 96%. Addition of antimurine IL-6 to murine supernatants abolished proliferative activity in the B9 assay by 97%.

Measurement of monokines by ELISA

While monokine bioactivity was significantly reduced with aging at various time-points, measurement of IL-1α, TNFα, and IL-6 in the same CFA-elicited macrophage supernatants by ELISA revealed no significant differences (Table 1). However, we did find a trend toward decreasing levels of all three monokines with increasing age. Overall, monokine levels as measured by ELISA were markedly lower than TNFα, IL-1, and IL-6 levels measured by bioactivity assays at 24, 8, and 4 h, respectively.

Effect of aging and eliciting agents on adherent cell numbers

Numbers of peritoneal exudate cells obtained following elicitation with either CFA or TG did not differ significantly among the three age groups (Table 2). In contrast, nonelicited resident peritoneal exudate cell yields were significantly greater from aged mice when compared with cell yields from young and middle aged mice. The percentage of cells which

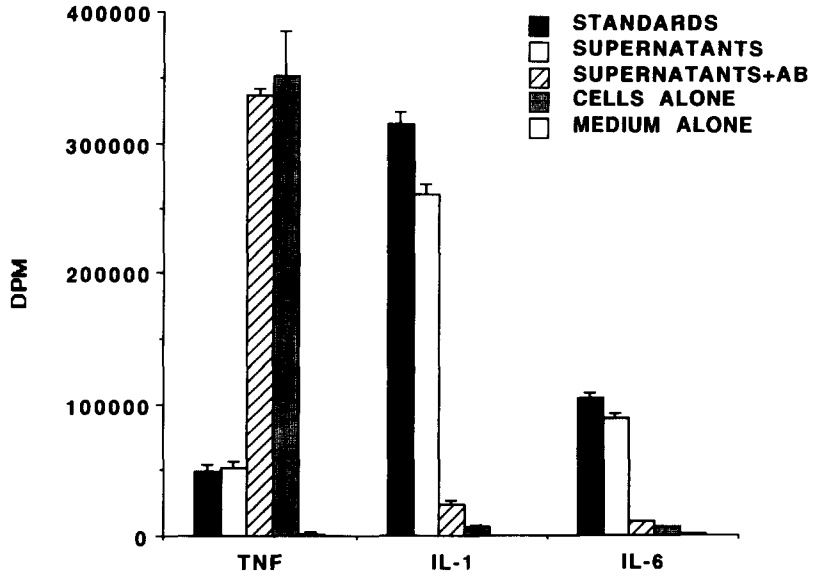

FIG. 4. Results of blocking and assay control experiments. Complete medium and WEHI 164 subclone 13 cells, D10.G4.1 cells, and B9 cells in complete medium were run as negative controls in all respective bioassays for TNF, IL-1, and IL-6. Standards used were recombinant human TNFα, concensus human IL-1, and recombinant human IL-6; activity was similar to that of the murine supernatants tested. Blocking experiments were done with antimurine TNFα mixed with supernatants from 5 different mice, antimurine IL-1 mixed with supernatants from 2 different mice, and antimurine IL-6 mixed with supernatants from 3 different mice. Data are expressed as the mean ± SE.

were macrophages, identified by morphology and staining with nonspecific esterase, did not differ significantly with age in exudates elicited with CFA or TG. However, the percentage of cells which were macrophages was significantly higher in young mice when nonelicited resident peritoneal macrophages were enumerated. The total number of cells which adhered to tissue culture plates were similar in all age groups, regardless of whether they were elicited with CFA or TG or were nonelicited resident cells. The percent of adherent cells that were esterase positive was greater than 95%, regardless of age or whether the cells were elicited with TG or CFA or were resident cells.

TABLE 1. CONCENTRATIONS OF MONOKINES IN SUPERNATANTS FROM CFA-ELICITED MACROPHAGES MEASURED BY ELISA

	TNFα		*IL-1α*		*IL-6*	
Age group	(N)	*(pg/ml)*	(N)	*(pg/ml)*	(N)	*(pg/ml)*
Young	(8)	3250 ± 462	(8)	120 ±24	(9)	1272 ± 325
Middle	(7)	2907 ± 269	(7)	91 ±8	(5)	893 ± 422
Aged	(8)	2629 ± 627	(9)	69 ±19	(8)	570 ± 148

Supernatants from CFA-elicited macrophages were incubated with LPS 10 μg/ml for 4 h (IL-6), 8 h (IL-1α), and 24 h (TNFα). All data are expressed as mean ± SE. Differences among age groups for each monokine were assessed by one-way analysis of variance, and no significant differences were found.

TABLE 2. TOTAL YIELD OF ELICITED AND RESIDENT PERITONEAL CELLS, PERCENT MACROPHAGES, AND ADHERENT CELL NUMBERS FROM THREE AGE GROUPS OF MICE

Cells[a]	*Age*	*TG*	(N)	*CFA*	(N)	*Resident*	(N)
Total yield (10^6)	Young	45 ± 4	(18)	54 ± 6	(9)	2 ± 0.2	(12)
	Middle	54 ± 6	(15)	50 ± 6	(7)	5 ± 1.1	(11)
	Aged	52 ± 8	(15)	67 ± 12	(7)	12 ± 2.0	(11)*
Macrophages (%)	Young	93 ± 1	(8)	89 ± 3	(4)	72 ± 3	(20)**
	Middle	89 ± 2	(6)	91 ± 1	(5)	49 ± 5	(9)
	Aged	96 ± 3	(4)	88 ± 1	(6)	54 ± 7	(8)
Adherent cells (10^6)	Young	0.8 ± 0.04	(8)	0.9 ± 0.1	(9)	0.9 ± 0.1	(11)
	Middle	0.7 ± 0.05	(6)	1.0 ± 0.1	(7)	1.3 ± 0.1	(11)
	Aged	0.9 ± 0.05	(4)	0.9 ± 0.1	(7)	1.3 ± 0.1	(11)

[a]Each data point represents the mean ± SE.
*Aged mice yielded more resident cells than young or middle aged mice, $p = 0.0001$ by one-way analysis of variance. **Young mice yielded a greater proportion of macrophages than aged or young mice, $p = 0.001$ by one-way analysis of variance.

DISCUSSION

Various approaches to assess the effect of aging on the monocyte–macrophage system have been used. Early studies of monocyte–macrophage function focused on the pyrogenic properties of the monokines (Kauffman, 1986; Norman *et al.*, 1988). Supernatants from shellfish glycogen-elicited rat peritoneal macrophages showed no decline in pyrogenic activity with increasing age (Kauffman, 1986). In contrast, a significant decline in the pyrogenic activity of supernatants was found with increasing age when murine adherent peritoneal cells were elicited with thioglycollate (Norman *et al.*, 1988).

Subsequent approaches to assess the effects of aging on monokines involved measuring their proliferative or cytotoxic effects on various cell lines. Inamizu *et al.*, using supernatants from LPS-elicited, adherent mouse peritoneal cells, showed a significant reduction in IL-1 mediated thymocyte proliferation with increasing age (1985). Bruley-Rosset and Vergnon found similar results using supernatants from resident nonelicited adherent murine peritoneal cells in a thymocyte proliferation assay (1984).

However, no significant differences were found when supernatants from nonelicited resident adherent rat peritoneal macrophages were assessed for IL-1 bioactivity in a thymocyte proliferation assay (Rosenberg *et al.*, 1983). In contrast, we found a decline in IL-1 and TNF bioactivity, by thymocyte proliferation and L929 cytotoxicity assays, respectively, in supernatants from shellfish glycogen-elicited rat peritoneal macrophages (Bradley *et al.*, 1989). These studies were corroborated by Davila *et al.*, who found a decline in TNF bioactivity by the WEHI assay in supernatants from aged rat resident peritoneal macrophages (1990).

In the above studies, investigators used various animal models and different methods of obtaining and stimulating macrophages. In our current study, we found that the eliciting agents used greatly influenced the occurrence of age-related differences in monokine bioactivity. Our results with nonelicited resident macrophages confirm similar studies with resident macrophages by Bruley-Rosset and Vergnon showing that IL-1 bioactivity is reduced in supernatants from aged mice (1984), and support findings by Davila *et al.* that TNF bioactivity is reduced in supernatants from aged rats (1990).

Monokine production in aged TG-elicited peritoneal macrophages contrasted greatly with the findings using nonelicited resident macrophages and CFA-elicited macrophages, and with prior studies of LPS-elicited (Inamizu *et al.*, 1985) and shellfish glycogen-elicited macrophages (Bradley *et al.*, 1989). When macrophages were elicited with TG, no apparent defect in monokine bioactivity was seen with aging; whereas aged macrophages elicited with LPS, glycogen, and CFA all showed a decline in monokine bioactivity (Inamizu *et al.*, 1985; Bradley *et al.*, 1989).

It is likely that the use of either resident or elicited peritoneal cells can greatly influence the outcome of experiments in aging, macrophage function, and cytokine production. Peritoneal macrophages elicited with TG have been found to have increased pinocytotic and recruitment rates, differences in protein synthesis patterns, and increased tumoricidal activity, when compared with proteose peptone-elicited macrophages (Edelson *et al.*, 1975; Hopper, 1986; Tannenbaum *et al.*, 1987; Higuchi *et al.*, 1990). Thioglycollate has also been associated with a depressive effect on microbicidal capacity (Spitalny, 1981; Leijh *et al.*, 1984). Direct comparisons of the effects of various eliciting agents (i.e., "stimulants or irritants" or activating agents) on monokine production in aging animals have not been carried out previously.

Differences in monokine bioactivity seen with CFA and TG might be explained on the basis of differences in the functional state of the macrophages elicited or heterogeneity of the populations elicited (Melnicoff *et al.*, 1989; Reed and Burnham, 1991). Interactions of these macrophages with other types of cells and cytokines in vivo also could alter monokine production in vitro. Differences in monokine production, seen with the use of different eliciting agents to obtain macrophages, might be explained by the induction of substances which alter macrophage function, such as prostaglandin E_2 (Goodwin and Messner, 1979) or other cytokines, such as interferon-γ (Collart *et al.*, 1986) interleukin-4 (Essner *et al.*, 1989), interleukin-10 (De Waal *et al.*, 1991), or granulocyte–macrophage colony stimulating factor (Heidenreich *et al.*, 1989). Similar hypotheses have been raised by Marcinkiewicz, who found that supernatants from TG- or LPS-elicited murine macrophages yielded differences in IL-1, IL-6, and TNF bioactivity (Marcinkiewicz, 1991; Tachibana *et al.*, 1992).

The function of aged macrophages has been assessed using primarily peritoneal macrophages (Perkins, 1971; Johnson *et al.*, 1978; Finger, *et al.*, 1982; Wustrow *et al.*, 1982; Petrequin and Johnson, 1984; Lavie and Gershon, 1988; Chen *et al.*, 1991) and only occasionally alveolar macrophages (Esposito *et al.*, 1988) or Kupffer cells (Caperna and Garvey, 1982). These studies have been performed with peritoneal macrophages elicited by a wide variety of different agents. In only two studies was the function of elicited macrophages compared with the function of nonelicited resident macrophages (Lavie and Gershon, 1988; Chen *et al.*, 1991). Although an age-related difference was seen in phagocytosis, superoxide generation, and microbicidal capacities in elicited macrophages, no age-related decrease was noted when nonelicited resident macrophages were used (Lavie and Gershon, 1988; Chen *et al.*, 1991).

We have also demonstrated that while monokine bioactivity was reduced with increasing age in supernatants from CFA-elicited cells, similar results were not found when the supernatants were assayed by ELISA methods. One possibility to explain these results is that IL-1, TNF, and IL-6 could have been produced in normal quantities, but inhibitors of IL-1 (Mazzei *et al.*, 1990; Svenson *et al.*, 1990), TNF (Seckinger *et al.*, 1988; Peetre *et*

al., 1988), and IL-6 (Novick *et al.*, 1989) have been described. Future experiments will have to address whether any of these inhibitors of cytokine activity are increased with aging.

Our studies suggest that the regulation of monokine secretion may be altered, but not necessarily defective in the aged. This concept echoes that of Russo *et al.*, who propose that aging does not cause clear-cut deficits in specific immune system function, but rather immune dysregulation leading to inappropriate responses by the aged host (1990). Our model may be useful in further studies of how aging alters homeostatic mechanisms which control monokine production.

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