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Calorie restriction inhibits the age-related dysregulation of the cytokines TNF-α and IL-6 in C3B10RF1 mice

Carolyn C. Spaulding, Roy L. Walford, Rita B. Effros*

Department of Pathology and Laboratory medicine, University of California School of Medicine, Center for the Health Sciences, Los Angeles, CA 90095-1732, USA

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Abstract

TNF-α and IL-6 are generally increased in the sera of aged humans and mice. The dysregulation of these cytokines may be critical in autoreactivity and immune dysfunction. In earlier studies we demonstrated that production of TNF-α and IL-6 following in vitro stimulation of peritoneal macrophages by LPS was reduced in old compared to young mice, and that dietary caloric restriction (CR) had no effect on the induction of TNF-α in this system. In the present study we examined the effects of age and calorie restriction on the constitutive production of both TNF-α and IL-6. Serum levels of both cytokines were significantly higher in old versus young mice. However, in old mice subjected to long term CR the serum levels were comparable to those of young mice. The potential involvement of normalization of TNF-α and IL-6 levels in the life extension effect of CR are discussed. © 1997 Elsevier Science Ireland Ltd.

Keywords: Caloric restriction; IL-6; TNF-α

* Corresponding author. Tel.: +1 310 8250748; fax: +1 310 2065178; e-mail: reffros@pathology.medsch.ucla.edu

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1. Introduction

As soluble mediators involved in many biological processes, cytokines are a major communication link both within the immune system and between the immune system and other organs [1]. During aging, a shift occurs in the ratio of naive to memory T cells, with an associated change in cytokine profile [2,3]. In particular, there is an increase in the elaboration of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), gamma interferon (INF-γ), and transforming growth factor-beta (TGF-β) [4,5].

TNF-α and IL-6 are potent multifunctional cytokines that share similar roles in exerting diverse effects on the immune system. They are both cellular regulatory molecules produced by lymphoid and non-lymphoid cells in response to a variety of stimuli. TNF-α is a 17 kDa protein produced in response to mitogens, bacteria, viruses, parasites and other cytokines. Its biological roles include anti-tumor activity, potentiation of CTL-mediated lysis of virally infected cells, immune modulation, induction of endothelial cell adhesion molecules and stimulation of IL-6 production. Thus, TNF-α plays a role in such diverse activities as hematopoiesis, inflammation, septic shock, infection and immunity [6,7].

IL-6, a 26 kDa protein also possesses pleotropic activity, playing a central role in host defenses and acting upon a wide variety of tissues. It influences such diverse processes as immunoglobulin secretion in B-cells, production of acute-phase proteins in liver cells, maturation of megakaryocytes and neuronal cells, differentiation of cytotoxic T cells, and inhibition of the growth of myeloid leukaemic cell lines. Over-production of IL-6 has been linked to AIDS and to age-associated disorders such as lymphoid malignancies, uterine cervical carcinoma, Castleman's disease, rheumatoid arthritis and systemic lupus erythematosis [6,8].

While surprisingly little is known about the physiological control of TNF-α or IL-6 under normal conditions, the genes of both cytokines are tightly regulated so that at least in healthy young animals, little of either cytokine can be detected in sera. However, evidence suggests that even in the absence of inflammation or other pathologic stimuli, serum levels of both TNF-α and IL-6 tend to be increased with advancing age [9–12].

It is well documented that dietary caloric restriction (CR) without malnutrition retards aging, extends maximum life span, and decreases the incidence of cancer and other age-associated diseases in mice, rats, and hamsters [13–15]. Unlike their control-fed counterparts which appear aged, calorically restricted mice appear physically youthful, are very active and show less external evidence of disease. For most parameters, CR has been shown to normalize dysregulated immune function or reverse immune decline in old mice. In a prior study, we demonstrated that CR had no effect on the production of TNF-α or IL-6 by peritoneal macrophages, isolated from mice of different ages following in vitro stimulation with lipopolysaccharide (LPS) [16]. In the present study, designed to test the effect of long term CR on uninduced in vivo levels of TNF-α and IL-6, we compared the levels of both cytokines in the sera of control-fed (CF) young, old, and calorically restricted old mice of the long-lived C3B10RF1 hybrid strain.

2. Materials and methods

2.1. Animals

Mice were bred and housed in the UCLA vivarium in an isolated unit designated for aging studies. All mice used in our studies are free of infection. To monitor for infection, sentinal mice are kept in each room of our semi-barrier facility. Serum samples are screened every six months for antibody titers against eleven common pathogens. Female progeny of the long-lived F1 hybrid strain (C3B10RF1), obtained from the cross between C3H.SW/Sn females and C57BL/10.RIII/Sn males, weaned at 28 days and individually caged, were randomly assigned to one of two dietary groups, either 50% calorie restricted or control fed (90% of ad libitum diet) [17].

2.2. Dietary regimen

Diets were minor modifications of the previous diets used in our laboratory for CR studies [17]. The diet was made 'nutrient-dense' by increasing all non-carbohydrate components by a ratio of 7:4. Restricted mice were fed once every other day and once on the weekend. The control mice were fed once daily and a one time double portion on the weekend [18].

2.3. Sample procurement

Only results from healthy-appearing mice, who maintained this status for 2–3 weeks following blood sampling and showed no significant recent weight loss, were included in the study. 0.5–1.0 ml of blood obtained from the retro-orbital sinus of each mouse was allowed to clot at room temperature, placed at 4°C for 2 h to allow clot retraction to increase serum recovery and centrifuged at 1200 rpm for 20 min. At least 75 μl of serum was removed from each sample, placed in a sterile tube, tested immediately or store at -70°C.

2.4. TNF-α and IL-6 studies

TNF-α and IL-6 levels were measured using commercial ELISA kits (Genzyme Diagnostics) following the manufacturers' recommended procedure and quantified by an ELISA reader (450 nm).

2.5. Statistical analysis

Data are reported as the mean $\pm$ S.E.M. Serum TNF-α and IL-6 data were compared by two-way ANOVA, followed by t-tests on pairs of data of interest. TNF-α and IL-6 concentrations were determined by linear regression of absorbance reading data points from TNF-α and IL-6 commercial standards.

3. Results

3.1. Effect of caloric restriction on the constitutive production of TNF-α and IL-6 in mice

A cross sectional study was done measuring uninduced circulating levels of TNF-α and IL-6 in the sera of young-adult CF (8–14 months), old CF (28–36 months), and old CR (28–36 months) mice. Mean TNF-α and IL-6 levels ± S.E.M. are shown in Figs. 1 and 2. Levels of both cytokines were significantly lower in sera from old CR mice compared to old CF mice ($P < 0.05$), and approximately the levels seen in young-adult CF mice. As none of the mice selected for these studies showed signs of disease, the results suggest that both TNF-α an IL-6 are dysregulated in aged mice, and that long term CR may inhibit or delay this dysregulation.

As shown in Table 1, large intra-group variations in the concentration of TNF-α and IL-6 were observed within the old CF cohort. However, the mean levels of both cytokines were significantly higher than those of the old CR and young-adult CF mice ($P < 0.05$). Approximately one third of all mice tested was sacrificed to

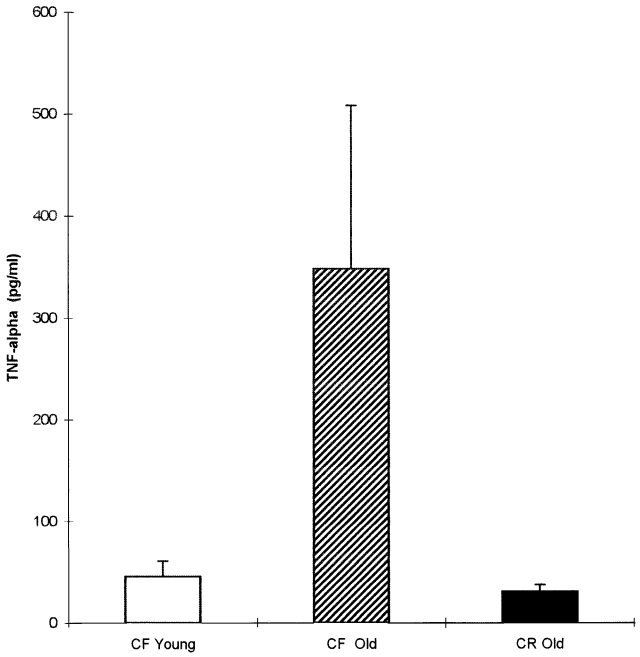

Fig. 1. Age-associated increases in serum levels of TNF-α are normalized by caloric restriction. Circulating levels of TNF-α in sera of CF young (8–14 months) mice and CF and CR old (28–36 months) mice are depicted in this histogram. This graph represents data from seven experiments. Data are reported as mean levels of TNF-α ± S.E.M. TNF-α levels in the sera of CF old mice (hatched bar) were significantly higher than that in CR old mice (shaded bar) ($P < 0.05$) and in CF young mice (open bar) ($P < 0.05$). (CF, control-fed; CR, caloric restriction).

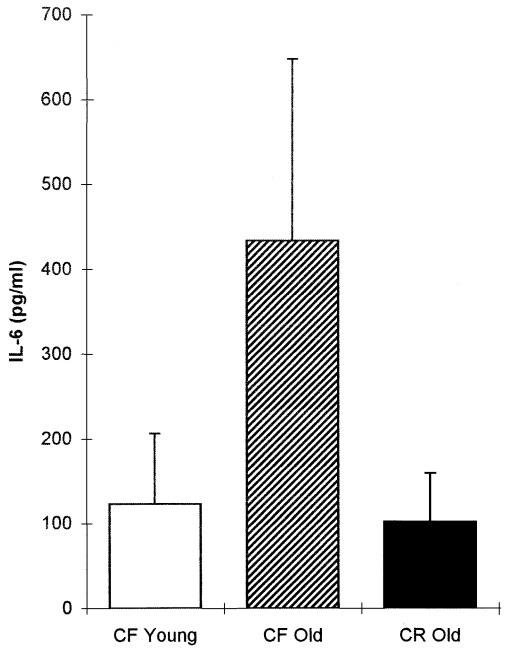

Fig. 2. Effects of age and caloric restriction on the constitutive production of IL-6 in CF young (8–14 months) mice and in CF and CR old (28–36 months) mice. These data represent the combined results of four experiments. Results are presented as the mean levels of IL-6 ± S.E.M. IL-6 levels in sera of CR old mice (shaded bar) were significantly lower than that in control fed old mice (hatched bar) ($P < 0.05$) but similar to that in CF young mice (open bar). (CF, control fed; CR, caloric restriction).

obtain organs for other experimental uses, and no evidence of internal disease or tumors were observed in CF young or CR old mice. The three CF old mice found to have tumors in the spleen and peritoneal cavity were omitted from the study and replaced with tumor-free mice from the same group. Unlike results from the old CF mice, levels of TNF-α (9–81) and IL-6 (2–430) from the old CR group consistently fell within a range similar to that of the CF young mice (13–87; 5–482 respectively).

4. Discussion

Current interest in TNF-α and IL-6 reflects the finding of an increase and general dysregulation in both cytokines in many age-associated diseases in humans, rodents, and a variety of other species. Ershler et al. [19] reported increased levels of IL-6 in plasma from healthy aged humans, monkeys and mice. Also in their study, mononuclear cell cultures from the lymph nodes of old rodents were shown to produce higher levels of IL-6. This change was delayed in rodents subjected to adult-onset CR. Volk et al. [20] reported that in both cross-sectional and longitudi-

nal studies, basal levels of IL-6 were increased in both control-fed and CR mice of the C57/BL6 strain. However, the greatest enhancement of IL-6 production was found to be associated with the presence of lymphoma, in both control-fed and CR old mice (18–25 months). Also, the increase in IL-6 levels in both the control-fed and CR old mice (25 month) were greater than those in 18 month old control-fed mice, and large variations in the range of IL-6 levels were also observed.

Daynes et al. [21] showed that peripheral blood mononuclear cells from healthy aged humans and splenocytes from old mice spontaneously produced IL-6 in vitro under serum free conditions, whereas cells from young humans or mice did not. Hotamisligil et al. [22] recently demonstrated a correlation between increased levels of IL-6 and insulin resistance. They showed that TNF-α is over-produced by adipose tissue in obese rodents and humans, and that TNF-α suppresses the action of insulin by inhibiting insulin receptor signaling. Han et al. [23] showed that in vitro production of TNF-α by peritoneal macrophages but not splenocytes increased significantly with age. Earlier studies in our own laboratory [16] examined the effects of CR on peritoneal macrophages from mice of different ages. We found that peritoneal macrophages from control old mice stimulated in vitro with lipopolysaccharide (LPS) showed reduced production of both TNF-α and IL-6. CR had no effect on this induced production of TNF-α by macrophages.

Our data confirm that levels of TNF-α and IL-6 increase with age, and that CR inhibits this increase. Throughout our cross-sectional study, CF old mice constitutively produced higher serum levels of TNF-α and IL-6 than CR old mice. Of the 54 CF old mice tested for TNF-α, 69% showed elevated serum levels; for IL-6, 60% of the 30 CF old mice tested had increased levels. The corresponding percentages

Table 1
A statistical summary of serum levels of TNF-α (a) and IL-6 (b) in the sera from young and old mice respectively

	CF young	CF old	CR old*
(a) TNF-α			
Number of nice tested	38	54	36
% Positive	24	69	31
Pg/ml ($\pm$S.E.M.)	45.5 ($\pm$15)	347.9 ($\pm$160)	30.3 ($\pm$7)
Median	34.9	39.5	23.1
Range	9–151	2–4716	13–87
(b) IL-6			
Number of nice tested	16	30	19
% Positive	31	60	42
Pg/ml ($\pm$S.E.M.)	122.9 ($\pm$83)	433.9 ($\pm$214)	102.2 ($\pm$57)
Median	10	85	34.7
Range	2–430	2–3175	5–482

The percent positive samples, mean levels, and range of both TNF-α and IL-6 from dietary calorie restricted old mice were significantly different from that of control fed (CF) old mice but similar to that of CF young.

* $P<0.05$ compared with CF old mice by t-test:ANOVA.

for TNF-α and IL-6 in CR old mice were 31 and 42% respectively. The large variation in the seven ranges of both cytokines in the CF old group was consistent with prior observations. In the CR mice, the concentrations of TNF-α and IL-6 fell within a more narrow range, and had significantly less variability than in the CF old mice. Our results also show that age-associated increases in TNF-α and IL-6 levels occur independently of each other, since for individual mice there was no correlation between these levels. Unlike the study by Volk et al. [20], the CR old mice used in our study were free of tumors as determined by external observations and autopsy. As depicted in Table 1, mean levels of both cytokines for CR old mice were similar to those measured in CF young mice. Interestingly, the range of body weights of the control-fed old mice (33–38 g) was at least 1.5 times greater than that of the CR old mice (22–25 g) and the level of TNF-α was significantly increased over the levels observed in the CR old mice. Thus, these data may support the findings of Hotamisligil et al. [22] that over expression of TNF-α from the adipose tissue promotes insulin resistance in obese rodents and humans.

Our results suggest that life-long CR induces a steady state of regulatory control of TNF-α and IL-6 production. Since calorie restriction decreases age-related diseases, reduces tumor incidence, improve immunity and increases life span, the normalization of TNF-α and IL-6 levels may be considered 'health enhancing'.

Acknowledgements

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