Cytokine production and aging: overproduction of IL-8 in elderly males in response to lipopolysaccharide

Jeanette A. Clark, Theresa C. Peterson*

Department of Medicine, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

Received 19 May 1994; revision received 17 August 1994; accepted 29 September 1994

Abstract

Interleukin-8 (IL-8) is a chemoattractant cytokine for polymorphonuclear neutrophils, and is found at the site of inflammation and infection. The levels of IL-8 from an elderly (ages 65–79) and young (ages 20–27) population were compared. Secretion of IL-8 was measured in monocyte conditioned medium (MCM), under both a spontaneous condition and with stimulation with detoxified LPS (10 mg/ml). Spontaneous production of IL-8 in the elderly group (39.4 ± 8.3 ng/ml, n = 16) was found to be significantly lower than the control group (66.4 ± 5.0 ng/ml, n = 17), P < 0.01. A sex difference was observed within the elderly population, with the male elderly producing 8.8 ± 2.1 ng/ml of IL-8 and the elderly females producing levels of 57.8 ± 9.1 ng/ml. There was a good correlation between IL-8 and IL-1 production in the elderly but differences between the elderly and young production of IL-1 did not reach statistical significance. IL-8 and TNF production did not correlate. Upon stimulation with LPS, the male elderly levels increased eightfold (70.1 ± 11.8 ng/ml) and was significantly different from the young male level, P < 0.01, while the female elderly showed no change with stimulation. No sex difference was observed in the control population. These results indicate that the spontaneous secretion of IL-8 in elderly males is lower than that of both elderly females and the young control group. However, upon stimulation with LPS, the elderly males are capable of an overproduction of IL-8 when compared to the young group and the elderly females. This overproduction may be the result of an in vivo priming in this healthy elderly group. The female elderly followed a pattern similar to the young group, showing no change upon stimulation with the detoxified LPS. Sex differences related to the immune system.
system have been noted in the past with females having a more active immune system, and these results may be related to this difference.

**Keywords:** Monocyte; IL-8; IL-1; TNF; Aging

1. Introduction

Aging has been defined as a decline in an individual's ability to adapt to environmental stress [1]. This decline has often been attributed to flaws developing within the immune system. Many different theories have been postulated regarding this change, ranging from degenerative changes related to wear and tear [2], autoimmune changes [1] as well as the possibility of preprogrammed cell death [3].

Decreases in both cell mediated and humoral immunity have been observed with age [4] but both a decrease and no change in granulocyte function have been reported [1]. Decreases in T-cell function have been well documented [1,2,5] showing decreases in proliferative capacity, IL-2 production and changes in the T-cell subsets. There are B-cell changes with age including decreased proliferative response, decreased antibody response to antigens such as tetanus toxoid, Candida or influenza vaccine and increased antiidiotype antigen and autoantibodies [5] which may play a role in the increase in autoimmune disease in the elderly [6,7]. Natural killer (NK) cells, which primarily attack tumour and virally infected cells, show increased activity with age and may be due to interferon gamma (IFN) activity [8].

The role of cytokines in the immune system is to provide a communication network between the different cells, allowing complex signals to be transmitted [9]. Tumour necrosis factor (TNF) and interleukin-1 (IL-1) are produced by many different cell types including T-cells and monocytes and they have varied functions ranging from being pyrogenic (IL-1) to tumouricidal activity (TNF).

Interleukin-8 (IL-8) is a chemoattractant for PMN and now has been linked with many different conditions including ARDS, rheumatoid arthritis and psoriasis. IL-8 is secreted by a variety of cells including macrophages, monocytes, fibroblasts and endothelial cells [10]. Its secretion has been linked to TNF and IL-1 levels [11,12]. IL-8 has been associated with many disease processes especially collagen and vascular disease including systemic sclerosis [13], psoriasis [14], as well as inflammatory bowel disease [15]. IL-8 is present in states of chronic infection [13] and acute infection [15].

The functions of IL-8 include polymorphonuclear neutrophil (PMN) chemotaxis and degranulation as well as the contraction of bronchial smooth muscle and inhibition of leukocyte adhesion to activated endothelial cells [10,16]. These activities appear to be opposing and may be mediated by two different forms of IL-8 [17]. IL-8 regulation is multifactorial with control exerted by other cytokines [18] and cells, such as erythrocytes [19] and PMNs [20].

Lipopolysaccharide (LPS) stimulates IL-8 secretion [21] either in vivo or in vitro, but LPS also appears to inhibit polymorphonuclear neutrophils (PMN) chemotaxis induced by IL-8 [22]. LPS is found on the surface of gram negative bacteria and its
adverse effects appear to be mediated by cytokines [23-25]. Macrophages and monocytes, when exposed to LPS, change from a resting scavenger form to become tumouricidal and antimicrobial. For this to occur the cells must have been previously primed by the cytokine, interferon gamma, allowing them to be sensitized to molecules such as LPS [26,27]. The LPS molecule is comprised of three parts: an oligosaccharide side chain, a core polysaccharide and Lipid A. Lipid A is a disaccharide with two phosphoryl groups and five or six acyl chains and has been determined to be the active part of the LPS molecule [23,28]. The monophosphoryl form of Lipid A has been referred to as 'detoxified endotoxin' [29]. This form is capable of eliciting a biological response but is not as toxic as LPS and Lipid A [30]. This study was designed to examine the spontaneous and stimulated production of IL-8 in the healthy elderly compared to the healthy young.

2. Materials and methods

2.1. Monocyte isolation and monocyte conditioned medium (MCM) preparation

Peripheral blood was taken from two populations, an elderly group, aged 65–79 years and a young control group, 20–27 years. The members of the study group were asked to complete a questionnaire including information such as sex, age, medications, disease status, recent infections, pregnancy, cigarette smoking and alcohol consumption. The elderly group consisted of 15 non-smokers and one light smoker (male) and 12 who drank no alcohol and four who ranged from very little to light (social) alcohol consumption. The age range of elderly females was 66–79 with a mean of 71.9 years and the age range of elderly males was 65–78 with a mean of 71 years.

The blood was taken by vacutainer tubes containing EDTA as an anticoagulant. It was then combined with cold RPMI-1640 (20 ml of blood per 30 ml of RPMI). This was mixed carefully by inversion and was then layered on Histopaque-1077 (Sigma) in a 15-ml conical bottom centrifuge tube and spun at 400 × g for 30 min. The white opaque layer was collected and pooled in 15-ml centrifuge tubes, with RPMI being added to bring the volumes up to 15 ml. The tubes were centrifuged at 200 × g for 10 min. The supernatant was discarded and the remaining cell pellet suspended in Leibovitz (L-15) medium. The cells were then plated and cultured in a 37°C ambient air incubator for 24 h. After this time, the medium was replaced with fresh L-15 medium and the cells were incubated for an additional 24 h. After the second 24-h period, the media was collected and filtered through 0.22-μm Millex filters and stored at -70°C. The medium will now be referred to as a monocyte conditioned medium (MCM). A typical monocyte preparation yielded 6 × 10^5 monocytes per 10 ml of blood.

During this second incubation, the cells were subjected to two different experimental conditions. In the first of these, the monocytes were allowed to incubate without any additions to the medium, this constituted the spontaneous group. The second group had Lipid A, a detoxified form of LPS added at the start of this second 24-h incubation period, at a concentration of 5 μg of Lipid A per ml L-15, and this was referred to as the stimulated group.
2.2. Interleukin-8 assay

IL-8 levels were determined in the monocyte conditioned medium by an ELISA (R and D Systems). The procedure for this assay involved four stages and was carried out in microtiteration wells which were coated with a murine monoclonal antibody specific for IL-8.

The samples were added to the test wells and incubated at room temperature for 2 h. The IL-8 that was present became bound to the antibody which is coated on the well in a solid-phase. After this incubation, the wells were washed, thereby removing all other components that were present in the sample. The second step involved the addition of an IL-8 conjugate, a polyclonal antibody specific for IL-8 that was bound to the enzyme horseradish peroxidase. After a second 2-h incubation, the plate was washed again. At this time, any unbound antibody, along with its attached enzyme was removed. Substrate for the peroxidase enzyme was added and was incubated for 20 min. After this time, a stop solution, consisting of 2 N sulfuric acid, was added and the resulting colour change was read at 450 nm. The readings were then compared to a standard curve that had been obtained using known concentrations of IL-8. The colorimetric change was proportional to the amount of bound conjugate and therefore the amount of IL-8 in the sample.

The minimum detectable concentration of IL-8 in MCM is 4.7 pg/ml and this assay shows no crossreactivity (< 5 pg/ml) with IL-1 or TNFα and shows intraassay precision (5.4%) CV and interassay precision (7.3% CV).

2.3. Interleukin 1β and tumour necrosis factor α assays

IL-1β and TNFα levels in the MCM were determined by ELISA (R and D Systems). The procedure was the same as that for the IL-8 ELISA. The minimum detectable concentrations were 4.5 pg/ml for IL-1β and 4.8 pg/ml for TNFα and the kits showed no crossreactivity with the other two cytokines.

2.4. Alanine aminotransferase (ALT) assay

ALT activity was obtained by testing serum samples by a colorimetric assay using a Sigma ALT kit. The samples were added to a volume of alanine-α-KG substrate and incubated at 37°C. After this, a colour reagent was added to the test solution and it was reincubated. The stop solution added to the mixture, was 0.4 M NaOH and the absorbance was measured immediately after at 490 nm. The results were compared to a standard curve of ALT activity.

2.5. Statistics

Parametric statistical tests were used to analyse the data. The data was compared using a paired student’s t-test (spontaneous vs. stimulated) or unpaired student’s t-test for two variable statistical analysis (elderly vs. controls). Results were considered significant at \( P < 0.05 \).

3. Results

Our results show that the resting levels of IL-8 produced by a healthy elderly population (39.4 ± 8.3 ng/ml) were significantly lower (\( P < 0.01 \)) than those produced
by a young control group (66.4 ± 5.0 ng/ml) (Fig. 1). We also determined a sex difference within the elderly population (n = 16); the spontaneous production of IL-8 by elderly males (8.8 ± 2.1 ng/ml, n = 6) was significantly lower than the elderly females IL-8 concentrations (57.7 ± 9.1 ng/ml, n = 10), (Fig. 1) which were comparable to the young subjects (66.4 ± 5.0 ng/ml). The young subjects demonstrated no

![Graph showing IL-8 levels](image)

**Fig. 1.** Effect of age on spontaneous secretion of IL-8. This figure shows a production of interleukin 8 by monocytes isolated from a healthy elderly population compared to a healthy young population and compares males and females. The monocytes were isolated from blood as described in the Methods section and IL-8 production was assessed as described in the Methods section. This figure represents spontaneous production of interleukin-8. Interleukin 8 levels are expressed in ng/ml and each bar represents the mean ± standard error for 16 elderly individuals vs. 18 young individuals and also compares males and females: *statistically significant difference compared to the elderly group, P < 0.01; **statistically significant difference compared to elderly males, P < 0.01.
sex difference for IL-8 production with young males \((59.4 \pm 6.5, n = 11)\) vs. \((66.4 \pm 8.9, n = 9)\) for young females.

Upon stimulation with detoxified LPS, the total elderly group showed a significant increase in IL-8 (Fig. 2). The male elderly population showed a dramatic increase in IL-8 production \((70.1 \pm 11.8 \text{ ng/ml})\), (Fig. 2). The stimulated elderly males were significantly higher than the stimulated young group \((P < 0.05)\), (Fig. 2). The young subjects showed a decrease in their IL-8 production \((41.9 \pm 4.4 \text{ ng/ml, } n = 17)\) with LPS treatment while no change was observed with the female elderly population \((54.2 \pm 9.1)\) following LPS treatment.

The TNFα levels were not different between the two populations (elderly levels of TNFα \((35.5 \pm 3.6 \text{ ng/ml})\) vs. young levels \((50.8 \pm 10.6 \text{ ng/ml})\)) and IL-8 levels did not correlate with TNFα levels \((r = 0.2, P = 0.11)\). The levels of IL-8 produced by control did show a marked correlation with the level of TNFα produced \((P < 0.001)\) under stimulated conditions. The IL-8 concentrations showed a positive correlation with IL-1β concentration in the elderly and young groups \((r = 0.83, P < 0.01)\) yet no significant difference in IL-1β concentration was seen between these two populations, (Fig. 3) likely due to high variability in IL-1 levels between individuals within each population.

IL-8 measurement in a group of patients with biopsy proven liver disease and elevated alanine aminotransferase (ALT) activity showed a significant elevation in IL-8 in liver disease compared to normal subjects (Fig. 4). Measurement of ALT activity showed a significant difference \((P < 0.001)\) between the elderly \((2.86 \pm 0.28 \text{ I.U.})\) and the young populations \((9.19 \pm 0.96 \text{ I.U.})\) but both groups were within the normal range for ALT (Fig. 5). This study has now been extended to a larger sample size and results support the initial findings i.e. elderly group \((n = 34)\) produced IL-8 levels \((38.3 \pm 10.4)\) vs. young group levels of IL-8 \((78.1 \pm 10.5, n = 33)\).

4. Discussion

Our results show that a population of healthy elderly people produces a lower amount of interleukin-8 than a young control population. Further investigation of the elderly group indicated that the elderly males had significantly lower production of IL-8 than elderly females such that elderly females were similar to the young group. Upon stimulation with detoxified LPS, the IL-8 production by the total elderly group increased to concentrations comparable to the total young group. The young population showed no further increase in IL-8 production after the addition of the detoxified LPS.

The IL-8 production upon stimulation with the LPS can be interpreted in the following way. The results suggest that if a lower resting level of IL-8 is produced by these subjects \((< 30 \text{ ng/ml})\), they can be stimulated by the detoxified LPS, while those individuals producing IL-8 above this level may already be at a maximum production and therefore cannot be stimulated to produce higher levels of IL-8.

All of the men in the elderly group showed low resting levels of IL-8 and had increased production upon the introduction of the detoxified LPS. Two members of the female elderly group also produced IL-8 at a resting level of less than 30 ng/ml
Fig. 2. Effect of age on LPS stimulated production of IL-8. This figure shows the IL-8 production by monocytes of the elderly population previously described which have been stimulated with detoxified LPS lipopolysaccharide and each bar represents a mean ± standard error of 16 individuals. IL-8 production is expressed in ng/ml; * statistically significant difference compared to elderly group, $P < 0.05$. This figure also describes the IL-8 production by elderly males in the presence of detoxified LPS to stimulate IL-8 production where each bar represents the mean ± standard error of 6 individual males; ** statistically significant difference compared to unstimulated elderly male monocytes, $P < 0.01$. These results also describe the production of IL-8 by monocytes obtained from elderly or young males following stimulation with detoxified LPS where each bar represents the mean ± standard error of six elderly males compared to nine young males; *** statistically significant increase compared to elderly stimulated males, $P < 0.05$. 
and showed an increased production of IL-8 upon LPS stimulation. The remainder of the females in the elderly group had IL-8 levels similar to those in the control group. All members of the young group had high resting levels of IL-8 and did not exhibit an increased production upon stimulation with the detoxified LPS.

The results also suggest that there is a difference in IL-8 production between the elderly males and females. From the data, it was seen that the male elderly patients...
Fig. 4. Spontaneous production of interleukin 8 by monocytes of patients with liver disease compared to normal subjects. This figure describes the spontaneous production of interleukin 8 by monocytes as described in the Methods section. Eighteen individuals with biopsy confirmed liver disease were compared to 67 normal subjects. Each bar represents the mean ± standard error of the number of individuals shown in brackets. Interleukin 8 is expressed in ng/ml; *statistically significant increase compared to normal subjects, $P < 0.05$.

showed a very low resting production of IL-8 while the IL-8 levels in elderly females were comparable to the control groups. It was also noted that the male elderly, upon stimulation with the detoxified LPS, showed an overproduction of IL-8 surpassing that of the control population, while the female elderly showed no change in IL-8 production. There are reports about the influences of sex steroids on cytokine
Fig. 5. Alanine aminotransferase activity in the elderly compared to the young group. This figure shows the serum alanine aminotransferase activity assayed as described in the Methods section in elderly and young individuals. The ALT activity is expressed in international units. Each bar represents the mean ± standard error of 16 elderly compared to 18 young individuals; *statistically significant difference compared to the elderly group, \( P < 0.001 \).

production. Females also have a higher incidence of autoimmune disease, such as Systemic Lupus Erythematosus (9:1) and Hashimoto's thyroiditis (25–50:1). Various experiments show this difference may be directly related to the effects of progesterone, estrogen and testosterone on the release of IL-1 and other cytokines. Lehmann et al. [31] showed that testosterone, in physiologic and pharmacologic doses, was capable of diminishing the mitogenic response in monocytes stimulated by proliferative agents such as phytohemagglutinin M (PHA) and pokeweed mitogen
(PWM). However, both progesterone and estrogen at physiologic levels were found to increase IL-1 production by monocytes and macrophages [32,33]. It was also shown by these researchers that when estrogen and progesterone reach higher levels, such as those seen in pregnancy, they appear to suppress IL-1 production. This information agrees with clinical observations that the severity of autoimmune disease decreases with pregnancy. Little is known about sex steroids and IL-8 production but we have shown a direct correlation between IL-1 and IL-8 so it may be reasonable to assume that the sex steroids may also affect IL-8 production. Further studies are required to clearly define the role of sex steroids in IL-8 production.

Our results indicate that LPS stimulation resulted in overproduction of IL-8 in elderly male subjects. These results suggest that in vivo priming may have occurred. Interferon gamma is an important cytokine that has been shown to be a priming agent for macrophage and monocyte activation [26]. After cells have been exposed to interferon they are said to be ‘primed’ and capable of producing a strong reaction if stimulated with a second signal, such as LPS. The monocytes of the elderly male population may have been exposed to IFN gamma sufficient to induce in vivo priming. These results also suggest that these individuals may respond to levels of LPS that would be subthreshold for IL-8 production in the young group.

The results reported here for IL-8 levels in liver disease patients confirm earlier reports [34]. Liver disease is chronic and therefore its prevalence in the elderly is higher. ALT, a liver enzyme used as an index in liver injury, was assessed in all subjects to determine if IL-8 production was related to underlying liver disease. Results indicate normal ALT in all individuals.

If the male and female values are combined, they show less difference from the young group’s values, and this interpretation of the data may be misleading because the large difference within the male population (aged vs. young) would not be seen.

The results presented here suggest that aging may be an important factor in IL-8 production, specifically in the elderly male population. The elderly population was shown to produce low levels of IL-8, but this was found to be largely due to the elderly males. The response of the elderly males to LPS (to simulate infection) suggests that though basal production of IL-8 was low, they were capable of mounting an immune response to infection.

Acknowledgements

We would like to acknowledge the involvement of members of the Dartmouth Kiwanis ‘Golden K’ and the Dartmouth Seniors Centre. Thanks are extended to Gerriann Davidson for technical support and to Susan Moore for secretarial support. This study was supported by the Dalhousie Medical Research Foundation and a grant from the Medical Research Council of Canada.

References


