Diminished Acute Phase Response and Increased Hepatic Inflammation of Aged Rats in Response to Intraperitoneal Injection of Lipopolysaccharide

Christian R. Gomez, Claudio Acuña-Castillo, Claudio Pérez, Elías Leiva-Salcedo, Denise M. Riquelme, Gamaliel Ordenes, Kiyoko Oshima, Mauricio Aravena, Viviana I. Pérez, Sumiyo Nishimura, Valeria Sabaj, Robin Walter and Felipe Sierra

[1] Instituto de Ciencias Biomédicas (ICBM), Centro FONDAP de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile, Santiago.
[2] Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, USACH, Santiago.
[3] Facultad de Ciencias de la Salud, Universidad Diego Portales, Santiago, Chile.
[4] Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Chile, Santiago.
[5] Department of Pathology, Loyola University Medical Center, Maywood, Illinois.
[6] The Lankenau Institute for Medical Research, Wynnewood, Philadelphia, Pennsylvania.

Address correspondence to Felipe Sierra, PhD, National Institute on Aging, NIA/NIH, 7201 Wisconsin Ave, Suite 2C231, Bethesda MD 20892. E-mail: sierraf@nia.nih.gov

 Abstract
Aging is associated with a deterioration of the acute phase response to inflammatory challenges. However, the nature of these defects remains poorly defined. We analyzed the hepatic inflammatory response after intraperitoneal administration of lipopolysaccharide (LPS) given to Fisher 344 rats aged 6, 15, and 22–23 months. Induction of the acute phase proteins (APPs), haptoglobin, α-1-acid glycoprotein, and T-kininogen was reduced and/or retarded with aging. Initial induction of interleukin-6 in aged rats was normal, but the later response was increased relative to younger counterparts. An exacerbated hepatic injury was observed in aged rats receiving LPS, as evidenced by the presence of multiple microabscesses in portal tracts, confluent necrosis, higher neutrophil accumulation, and elevated serum levels of alanine aminotransferase, relative to younger animals. Our results suggest that aged rats displayed a reduced expression of APPs and increased hepatic injury in response to the inflammatory insult.

Key Words: Liver • Inflammation • Injury • Acute phase response • Aging

THE acute phase response (APR) comprises a group of inflammatory reactions induced by infection or tissue damage ([1]) that serve, in part, to limit further damage and to activate tissue repair mechanisms ([2],[3]). During the APR, the liver and other tissues respond to insult or injury by a dramatic change in the production of a variety of proteins, collectively referred to as acute phase proteins (APPs), the functions of which include modulation of

specific inflammatory responses and transport of metabolites and other molecules needed for tissue repair ([4]). Although the APR is nonspecific (part of the innate immunity), the pattern of expression of the APPs is relatively species-specific, so that in humans the primary APPs are C-reactive protein and serum amyloid A, whereas in the rat they are α2-macroglobulin, α1-acid glycoprotein, serum amyloid A, and haptoglobin ([5]).

Aging has been shown to be associated with a deterioration of the adaptive immune system ([6]). However, the effects of old age on the innate immune system, including the APR, are rather poorly defined at this time ([7]). Both aged humans and animals show elevated baseline levels of several circulating proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) ([8–10]). Among the proinflammatory cytokines, IL-6 is the most potent inducer of hepatic APPs ([1]). In addition to increased basal levels in aged individuals, elevated levels of IL-6 have been reported during the acute phase of elderly patients hospitalized with pneumonia ([11]), in older volunteers given endotoxin ([10]), and in animal models of inflammation ([12–14]). In fact, an increase in the circulating level of this cytokine has been proposed as a predictor of mortality risk in longitudinal studies of elderly cohorts ([15],[16]). Paradoxically, despite these increases in both basal levels and induction of IL-6 following an inflammatory challenge, decreased expression of APPs in response to inflammatory stimuli such as lipopolysaccharide (LPS) has been found in aged rodents ([17],[18]).

Although the molecular mechanisms responsible for the age-dependent changes in APR have not yet been elucidated, it has been shown that aging affects both the steady state and induced expression of transcription factors controlling the hepatic expression of APR genes ([19]). For example, in the aged mouse, hepatic transcription factors of the CCAAT/enhancer-binding protein (C/EBP) family present altered constitutive messenger RNA (mRNA) levels and binding activity ([20],[21]). Specifically, nuclear extracts from normal aged mouse livers have decreased p42C/EBPα levels and binding activity, whereas those of the inhibitory partners p20C/EBPα and p20C/EBPβ are increased ([20]). On induction by LPS, however, the induction of DNA binding activities of both C/EBPα and C/EBPβ is delayed significantly in aged mouse livers ([20]). Similarly, in nuclear extracts from the liver of aged rats, activator protein 1 and nuclear factor-κB, show increased binding activities in older animals ([22]), suggesting that the normal aging liver exhibits characteristics of a chronic inflammatory state even in the absence of stressors.

Whereas these changes offer a partial explanation for the incapacity of the aged liver to mount an effective APR and recover from an inflammatory insult, the contribution of hepatic pathology to these defects in advanced age has not been thoroughly explored. Under the hypothesis that the reduced expression of APPs during aging is associated with an elevated hepatic injury, we have administered a systemic inflammatory insult to Fisher 344 rats of different ages. Our data indicate that, in addition to a decreased responsiveness to circulating IL-6, the liver of aged rats suffers considerably more and longer pathological damage in response to the intraperitoneal (i.p.) injection of LPS.

METHODS

Animals

Male Fisher 344 rats of different ages were obtained from the National Institute on Aging colony at Harlan Laboratories (Indianapolis, IN). They were housed individually in a specific pathogen-free facility at the Lankenau Institute for Medical Research, and were fed a laboratory chow ad libitum. These rats have a median lifespan of 24 months, and were killed at the ages of 6 months (adult), 15 months (middle-aged), and 22–23 months (aged). The experimental protocols described here followed the guidelines established by the publication *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985) and were approved by the Lankenau Institute for Medical Research Institutional Animal Care and Use Committee.

Exposure to LPS

Four or five rats of each age received an i.p. injection of LPS at 2 mg/kg from *Pseudomonas aeruginosa*, serotype 10 (Lot: 99H4059, Sigma Chemical Co., St. Louis, MO), dissolved at 5 mg/mL in phosphate-buffered saline (PBS) under sterile conditions. The LPS dose used in these experiments did not produce mortality in any of the age groups. To avoid confounding factors related to circadian rhythms, all injections were given between 8 AM and 10 AM. Control rats received a similar volume of PBS as a carrier medium and were killed immediately. Animals given LPS were killed at different times ranging between 0.5 and 24 hours after the challenge. Serum was collected and the liver was flash frozen in liquid N_2 and kept at $-80°C$ until use.

RNA Extraction and Analysis

Total RNA was prepared from 100 mg of minced liver by the phenol–chloroform method ([23]), modified for carbohydrate extraction ([24]). Total RNA (5 µg) was electrophoretically fractionated on glyoxal gels ([25]), electrotransferred to Gene Screen membranes (Dupont/NEN Research Products, Boston, MA), fixed by exposure to ultraviolet light, and hybridized to random-labeled complementary DNA (cDNA) probes. Autoradiograms were developed and quantitated using a Bio-Rad Phosphorimager (Bio-Rad, Hercules, CA).

Serum Determination of APPs

Blood was collected and allowed to clot for 10 minutes. Serum was prepared by centrifugation at 2000 x *g* for 5 minutes, then immediately frozen at $-80°C$ until use. Serum samples were diluted 1:500 in PBS, and 10 µL of the resulting solution was resolved by 7.5% mini sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After electrophoresis and electrotransfer to nitrocellulose membranes, the blots were probed with antibodies against human haptoglobin (catalog number H8636; Sigma-Aldrich, St. Louis MO), human α1-acid glycoprotein (AGP; donated by Dr. Jack Gauldie, Ontario, Canada), and rat T-kininogen (T-KG; produced in our laboratory) diluted 1:2000, 1:1000, and 1:4000, respectively. A pooled serum sample obtained from adult rats after 6 hours of LPS administration was added as a loading control in each membrane to compare each of the blots.

Analysis of Serum Alanine Aminotransferase

Serum alanine aminotransferase (ALT) activity was assessed using a commercial kit

(Wiener Laboratories, Rosario, Argentina). Ten microliters of serum from each mouse was assayed for ALT, as recommended by the manufacturer. Data are reported as ALT units per liter of serum.

Liver Histology and Pathological Evaluation
Livers were fixed for 8 hours in 1% paraformaldehyde then embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E) for light microscopy. Ten fields per section were analyzed for neutrophils on the basis of their distinct morphology (granular cytoplasm with a multilobed nucleus), and accumulation was scored as neutrophil cells/mm^2. Hepatic inflammation was evaluated and scored by a pathologist in a blinded manner at 200x magnification. An inflammation score of – (no foci of inflammation), + (<2 foci of inflammation), ++ (2–4 foci of inflammation), or +++ (>4 foci of inflammation) was assigned.

Analysis of Serum IL-6 Levels
Determination of serum IL-6 levels was performed in samples from animals killed at 0.5, 1, 2, 4, 6, and 12 hours after LPS injection, or 0 hours after PBS injection. Measurements were performed by enzyme-linked immunosorbent assay (ELISA) with an OPTiEA kit (catalog number 2610KI for rat IL-6; BD Pharmingen, San Diego CA). The limit of detection for this kit is 78 pg/mL. Microplates were read in a microplate reader at 450 nm and at 570 nm for correction of optical imperfections.

Data Quantitation and Statistical Analysis
Quantitation of northern and western blots was performed by densitometric scanning. Analysis of variance and Tukey–Kramer multiple comparisons were used to determine statistical significance using the GraphPad Prism statistical package (version 2.0; San Diego, CA). A *p* value <.05 was considered significant.

RESULTS
Defect in Circulating Levels of APPs in Aged Rats
Circulating levels of APPs were analyzed by western blotting of the serum from rats of different ages given either PBS as control or LPS. Figure 1A shows that, in adult and middle-aged rats receiving LPS, serum haptoglobin levels increased in a similar fashion, becoming statistically significant by 12 hours. In contrast, in aged rats, induction of haptoglobin was only observed at 24 hours (*p* <.05, 14-fold when compared to age-matched controls). However, reduced levels of haptoglobin were observed in aged rats at 6 and 12 hours after LPS administration relative to those of adult and middle-aged animals (*p* <.05). No statistical difference in the levels of haptoglobin was observed at 24 hours after LPS injection. Induction of serum AGP levels (Figure 1B) was similar in all age groups at both 6 and 12 hours. However, at 24 hours, AGP levels were significantly lower in aged animals (48-, 16-, and 11-fold induction when compared to age-matched controls for adult, middle-aged, and aged rats, respectively; *p* <.05). Under basal conditions, circulating levels of T-KG (Figure 1C) were higher (3-fold, *p* <.05) in aged rats, as we have previously described

([26](#)). T-KG induction was observed in adult and middle-aged animals starting at 6 hours after LPS, reaching 5-fold at 24 hours ($p <.05$, compared to basal levels). In contrast, aged rats did not show significant variation in their level of serum T-KG relative to control animals until 24 hours after LPS injection, where only a 2-fold induction over control rats was observed ($p <.05$). Observed levels at this point were similar among the three experimental groups.
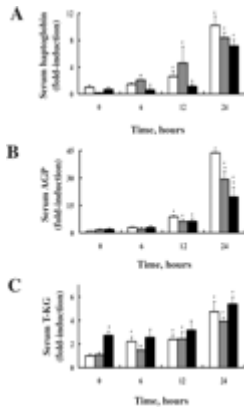

Figure 1. Induction of serum levels of acute phase proteins from lipopolysaccharide (LPS)-treated rats. Haptoglobin (**A**), human α1-acid glycoprotein (AGP) (**B**), and T-kininogen (T-KG) (**C**) levels were determined by western blot assay in serum dilutions obtained from adult (*white bars*), middle-aged (*gray bars*), and aged rats (*black bars*) at 0, 6, 12, and 24 hours after LPS treatment. Data are expressed as mean ± standard error of the mean of the fold induction over adult controls (given the value of 1). $n = 4–5$. [#]$p <.05$ from age-matched phosphate-buffered saline at 0 hours. *$p <.05$ from adults at the same time point
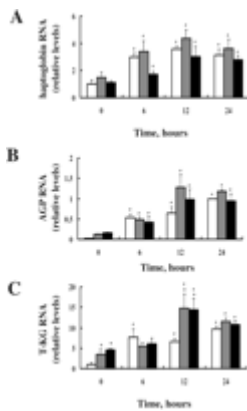
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Hepatic Expression of APP mRNAs
Total hepatic RNA was extracted, and northern blot analysis was used to assess the expression of the APP mRNAs ([Figures 2](#) and [3](#)). Densitometric quantitation shows that haptoglobin mRNA ([Figure 3A](#)) was induced by 3-fold at 6 hours after LPS administration in both adult and middle-aged rats, relative to their age-matched controls. At this same time point, aged rats displayed a discrete but not statistically significant increase in haptoglobin mRNA. In contrast, in aged rats haptoglobin mRNA peaked at 12 hours after LPS injection ($p <.05$). At this time point, as well as at 24 hours following LPS administration, similar induction of haptoglobin mRNA (about 3-fold) relative to controls was observed regardless of age. No age-related differences in the course of induction of hepatic AGP mRNA were observed among all experimental groups ([Figure 3B](#)). At 6 hours after LPS, a 5-fold induction of T-KG mRNA ([Figure 3C](#)) was observed only in adult rats. A similar fold induction of T-KG mRNA was observed in adult rats at 12 and 24 hours after LPS. Middle-aged and aged rats showed a 2-fold induction of T-KG mRNA at 12 hours following LPS injection. Induction levels observed at 24 hours were similar regardless of age.
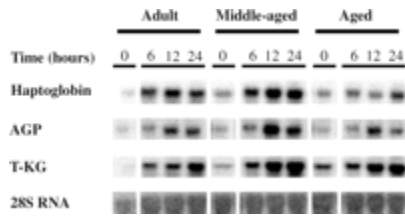
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Figure 2. Northern blot analyses on total liver RNA. Adult (6-month-old), middle-aged (15-month-old), and aged (22- to 23-month-old) Fisher 344 rats received an injection of either phosphate-buffered saline or lipopolysaccharide at 2 mg/kg and were killed at the indicated time points. Total RNA was isolated from the livers and subjected to northern hybridization analysis using a complementary DNA for each gene as a probe. Ribosomal 28S RNA was also measured for normalization. Each lane contained 5 µg of RNA derived from individual rats. AGP = human a1-acid glycoprotein; T-KG = T-kininogen

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Figure 3. Quantitation of hepatic expression of acute phase protein messenger RNAs (mRNAs) from lipopolysaccharide (LPS)-treated rats. Haptoglobin (**A**), human a1-acid glycoprotein (AGP) (**B**), and T-kininogen (T-KG; **C**) mRNA levels were determined by northern blot of total hepatic RNA obtained from adult (*white bars*), middle-aged (*gray bars*), and aged rats (*blacks bars*) at 0, 6, 12, and 24 hours after LPS administration. Data are shown as mean ± standard error of the mean, relative to the level observed in adult controls (0 hours) for both haptoglobin and T-KG. In the case of AGP, basal levels in adult animals were too close to the limit of detection of the assay; therefore, the level observed in adult animals 24 hours after LPS was used for quantitation. n = 4–5. [#]p <.05 from age-matched phosphate-buffered saline at 0 hours. *p <.05 from adults at the same time point

Effects of Aging on Serum Levels of IL-6

Our results show a defect in the expression of APPs. Because IL-6 is the most potent inducer of hepatic APPs ([1]), altered expression of this cytokine should be expected to be found in aged animals receiving LPS. Serum levels of IL-6 were analyzed and, irrespective of age, were undetectable in the serum of control animals and even at 1 hour after LPS ([Figure 4]). IL-6 was maximally induced at 2 hours after LPS administration in adult rats, and induction of this cytokine was similar at this time point for all age groups. In addition, middle-aged and most conspicuously aged rats, showed a further induction of this cytokine, with a maximum at 4 hours after LPS injection. Thus, at 4 hours following LPS, aged rats had a 2.5-fold higher level of serum IL-6 than that of younger experimental groups (p <.05). In addition, although circulating levels of IL-6 returned to almost basal levels by 6 hours in adult and middle-aged rats, serum IL-6 levels remained elevated in aged animals, even up to 12 hours after the insult. Overall, these results describe a persistent induction of serum IL-6 in aged rats receiving LPS, when compared to their younger counterparts. Additionally,

they show that increased serum levels of IL-6 in aged rats given LPS contrasts with defective expression of hepatic APPs.
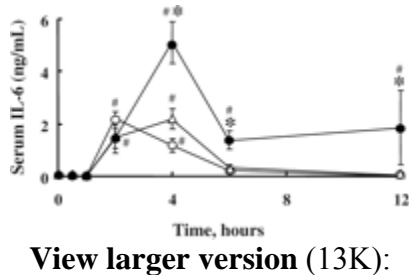

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Figure 4. Serum interleukin-6 (IL-6) from lipopolysaccharide (LPS)-treated rats. IL-6 levels were determined by enzyme-linked immunosorbent assay in serum dilutions obtained from adult (*white circles*), middle-aged (*white triangles*), and aged rats (*black circles*) at 0, 0.5, 1, 2, 4, 6, and 12 hours after LPS treatment. Data are shown in ng/mL as mean IL-6 ± standard error of the mean. $n = 4–5$. [#]$p <.05$ from age-matched phosphate-buffered saline at 0 hours. *$p <.05$ when comparing aged rats to other groups at the same time point

Histological Analysis of the Liver
To analyze the effects of aging on the hepatic inflammatory response after LPS administration, liver histology was evaluated. Figure 5 shows representative images of liver H&E-stained sections from both control animals and after 12 hours of LPS administration. In control adult and aged rats, inflammatory cells were rarely present in the portal tract (Figure 5, A and D) or in the hepatic parenchyma. After LPS injection, adult animals showed a slightly increased number of inflammatory cells in the portal tract (Figure 5B), with mild lobular inflammation, scant lobular spotty necrosis, and occasional clusters of neutrophils (Figure 5C). Middle-aged rats showed an infiltrate of a similar nature to the one seen in adult rats (data not shown). In contrast, in aged animals, a significant number of inflammatory cells were noted in both the portal tract and the hepatic parenchyma after injection of LPS (Figure 5E). In addition, confluent necrosis, recognized as zones of necrotic hepatocytes containing various inflammatory cells, was often observed (Figure 5F).
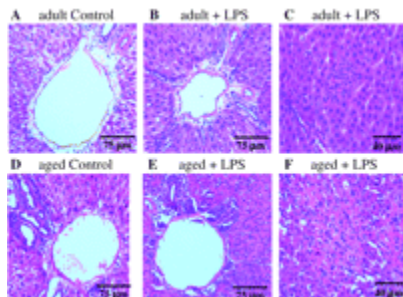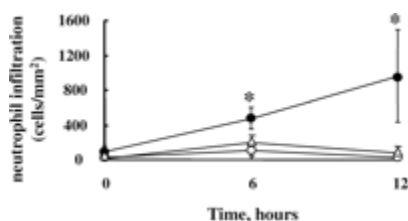

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Figure 5. Histological analysis of hepatic inflammation. Livers from adult and aged rats obtained at 0 or 12 hours after lipopolysaccharide (LPS) treatment were fixed. Five-micron sections of liver were stained with hematoxylin and eosin and evaluated by light microscopy. Representative sections of liver are shown from adults (**A–C**) or aged (**D–F**) rats, either control (**A** and **D**) or treated with LPS for 12 hours (**B, C, E,** and **F**). *Solid bars represent the scale, in micrometers*

To quantify the degree of hepatic inflammation, hepatic neutrophils were counted in H&E-stained sections obtained from control animals given PBS at 6 or 12 hours after LPS injection (Figure 6). Animals that received PBS injections did not show age-related differences (30 ± 18, 9 ± 5, and 97 ± 65 neutrophils/mm^2 for adult, middle-aged, and aged, respectively; not significant). LPS injection augmented the number of hepatic neutrophils present within the tissue irrespective of age. In adult and middle-aged rats, neutrophil numbers peaked at 6 hours. A 3-fold increase (not significant) was observed for young rats, and a 20-fold increment ($p < .05$) was seen in adult animals. Both age groups returned neutrophil accumulation to basal levels 12 hours after LPS administration. The time course of the hepatic accumulation in aged rats was quite different. It presented a continuous increase that was much higher than the one observed in younger groups (4-fold at 6 hours and 17-fold at 12 hours, $p < .05$). From these results it can be concluded that aged animals administered LPS undergo a more injurious hepatic inflammatory process compared to their younger counterparts.

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Figure 6. Quantitation of neutrophil accumulation in the liver of rats given lipopolysaccharide (LPS). Livers from adult (*white circles*), middle-aged (*white triangles*), and aged (*black circles*) rats obtained at 0, 6, and 12 hours after LPS administration were fixed, and 5-micrometer sections were stained with hematoxylin and eosin. The number of neutrophils in 10 fields was counted using a 60x objective. $n = 5–8$, data are shown as mean number of neutrophils per 10 fields $\pm$ standard error of the mean. *$p < .05$ when comparing aged rats to other groups at the same time point

Evaluation of Hepatic Injury

Liver injury can be identified by markers such as the appearance of necrotic foci in the hepatic parenchyma (27). Table 1 shows an evaluation of the number of necrotic foci per tissue section in control rats or after 6 or 12 hours following LPS administration. Livers from adult control rats showed absence of necrotic foci; however, a small number of necrotic foci was observed in middle-aged and aged control rats. At 6 hours following LPS injection, necrotic foci were observed in all age groups, relative to their age-matched controls. At 12 hours after LPS administration, adult and middle-aged rats had a reduced number of necrotic foci, relative to that observed at 6 hours after LPS. In contrast, the number of necrotic foci did not decrease in aged rats. These results suggest sustained hepatic injury in aged rats given LPS, relative to that seen in adult and middle-aged animals.

View this table:	Table 1. Histological Assessment of Liver Inflammation in Rats Given Either PBS or LPS.

The major source of ALT is the liver and, because of this, elevation in serum level of this enzyme has been used as an index of hepatic injury ([27](javascript:void(0)),[28](javascript:void(0))). Serum ALT levels were tested in control animals and 24 hours after LPS administration ([Table 2](javascript:void(0))). Detectable serum levels of ALT were found in all experimental groups. Animals given PBS did not exhibit significant differences in serum ALT regardless of age. LPS-treated adult and middle-aged rats did not show an elevation in serum ALT, relative to that of control animals, either at 24 hours ([Table 2](javascript:void(0))) or at 12 hours (data not shown). In contrast, aged rats given LPS displayed increased serum levels of ALT by 1.4-fold at 12 hours (data not shown), and 3.6-fold at 24 hours ([Table 2](javascript:void(0))) over the levels observed in aged control rats ($p < .05$). Overall, these results confirm that, under our experimental conditions, i.p. injection of LPS induces significant, sustained hepatic injury in aged rats, whereas younger animals are better able to recover from this insult.

Table 2. Serum ALT
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DISCUSSION

We have studied the effects of aging on the expression of APPs in Fisher 344 rats given LPS. Our results show that age affected serum levels and hepatic mRNA expression of representative APPs. Taken as a whole, both serum protein and hepatic mRNA induction was observed to be delayed and/or reduced in aged rats, compared to younger counterparts. Furthermore, in adult and middle-aged rats, maximum levels of IL-6, the major modulator of the APR, were observed at 2 hours. Serum levels of this cytokine were similarly induced in aged rats at this time, so the delay in induction of APR mRNA or proteins cannot be caused by differences in the level of IL-6. Surprisingly, however, maximal levels of IL-6 in aged rats were observed later, and were much higher than those observed in younger animals. A similar effect has been observed for other inflammatory mediators such as TNF-α (data not shown) and IL-1β ([12](javascript:void(0))). Despite this increase, the response in aged rats remained subdued. The exacerbated increase in IL-6 is likely to be either the cause or the result of the increased hepatic injury observed in these animals. Overall, our findings suggest that the reduced expression of APPs in aged rats administered LPS is associated with hepatic injury in response to the inflammatory insult.

Our results show that, as a function of age, there is defective expression of APPs following an LPS challenge. Relative to adult and middle-aged animals, aged rats given LPS have a delay in the induction of haptoglobin and T-KG, as well as a decreased level of AGP at 24 hours poststimulus. Although our results in serum might be unique to LPS-treated Fisher

344 rats, others have reported changes similar to those reported here in APP mRNA levels in the liver of aged mice ([17],[18]). Carter and colleagues ([17]) reported an age-related increase in the time required for AGP mRNA to respond to LPS in aged Balb/c mice. We did not observe a similar effect either at the mRNA or protein level in LPS-treated Fisher 344 rats. Similarly, a marginal reduction in haptoglobin mRNA in aged rats at 6 hours after LPS contrasted with a more prolonged defect in serum levels of this APP. A discrepancy between the mRNA and protein data for AGP at 24 hours after LPS could be explained by evidences describing age-related deficiency in the posttranscriptional regulation of hepatic APPs synthesized either under steady-state conditions ([29]) or in response to LPS ([20]). Adding to this complexity, T-KG mRNA induction was reduced as the result of age in rats given LPS, mainly as a result of an increase in basal levels. Despite this difference, age had no significant impact on T-KG serum protein other than to increase baseline.

The kinetics of IL-6 induction shows a complex pattern. IL-6 is the major inducer of type II acute phase genes such as haptoglobin and T-KG. In adult and middle-aged rats, induction of IL-6 was maximal at 2 hours, and this increase has been shown to be critical for induction of APP gene expression. In aged rats, however, induction of IL-6 at 2 hours was comparable to that observed in younger animals, and yet, induction of IL-6-dependent APPs at 6 and 12 hours was not significant in animals in this age group. This fact adds complexity to the interpretation of the so-called "pro-inflammatory" state of elderly individuals. Although serum levels of pro-inflammatory cytokines such as IL-6 are indeed often found to be elevated in healthy aged individuals ([15],[30]) or overly induced after an inflammatory stimulus, relative to young individuals ([12],[16],[31]), the results described here show that the hepatic response to these mediators—in terms of APP expression—appears to be blunted. It is possible that the increased levels of circulatory mediators actually occur as a response to the lack of responsiveness from target tissues such as the liver, much as the increased levels of insulin in type II diabetes appear to be the result of diminished tissue sensitivity to the hormone.

IL-6-dependent induction of acute phase genes requires the activation of several signal transduction pathways, some of which have been shown to be less active in the liver of aged individuals. For example, both the steady state and the activation of the Janus-activated kinase 2, and signal transducer and activator of transcription-3, are decreased in livers of aged mice ([32]). Similar results have been described for the mitogen-activated protein kinase, extracellular signal-regulated kinase (ERK), in hepatocytes from aged rats stimulated with epidermal growth factor ([33]), as well as for ERK and c-Jun N-terminal kinase in liver from aged rats given LPS (Walter R, Sierra F, 2000, unpublished results). These decreases could contribute to the lack of response to this cytokine.

In addition to these molecular and intracellular explanations, the possible role of hepatic inflammation and tissue injury has not been properly addressed. In young rats, a similar dose of LPS as used here produced neutrophil accumulation ([34]) with hepatocellular degeneration and coagulative necrosis in midzonal regions of liver lobules ([35]). As aged animals given an inflammatory challenge consistently show higher mortality relative to younger animals given the same stimulus ([12],[31]), we decided to keep i.p. LPS as the inflammatory challenge. Nevertheless, under our experimental conditions, some of the features depicting liver injury, such as a transient elevation in necrotic foci, were observed

to some extent in adult and middle-aged rats receiving LPS. However, hepatic injury was greatly exacerbated in aged rats, and in addition to the markers of hepatic injury shown in Figures 4 and 5 and Tables 1 and 2, we observed some signs of apoptosis—evidenced as DNA laddering at 12 hours after LPS, only in aged rats (data not shown).

In this study, the insult was administered at a distal site, the peritoneum. However, when activated by LPS, many factors occurring systemically, locally, or both could act additively to produce hepatic injury. In addition to the already discussed normal induction of IL-6 at 2 hours after LPS, aged rats (and to a lesser extent, middle-aged ones) showed a significantly enhanced induction of this cytokine at later times, with a marked peak at 4 hours, but serum levels remained high for at least 12 hours (Figure 4). Our studies do not allow the discrimination between cause and effect, but IL-6 has been shown to be causally related to inflammation and tissue injury, and conversely, it has also been shown that inflamed and injured tissues are capable of high levels of production of cytokines such as IL-6 (36–39). In contrast, antiinflammatory and hepatoprotective roles have also been proposed for IL-6 following acute injury in young rodents (40,41). However, recent results show improved survival and reduced hepatic injury in aged IL-6 knockout mice given an LPS challenge (31,42), relative to aged wild-type mice given the same insult. These observations, together with those reported here; suggest that the regulatory control of IL-6 in the inflammatory response is lost during aging and that elevated levels of this cytokine result in tissue injury.

It is anticipated that these studies could constitute the basis for therapeutic strategies aimed at avoiding liver injury and concomitant loss of function during the APR to systemic insult, not only for the aged population, but also for patients of all ages.

Acknowledgments

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