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Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice

Abstract

The different physiological roles of interleukin-1alpha (IL-1alpha) and interleukin-1beta (IL-1beta) are not well understood, especially when considering the apparent overlap and redundancy of the two IL-1 molecules. Characterization of IL-1alpha and IL-1beta expression was performed in this study in organs from young and old mice, using immunohistochemistry and ELISA (enzyme-linked

immunosorbent assay). The results indicate that organ IL-1alpha and IL-1beta display different patterns of expression: IL-1alpha is manifested more prominently in lymphoreticular organs (lungs, small intestine, spleen, liver), while IL-1beta is more evident in highly specialized and more vulnerable organs, which do not play a leading role in defense against infections and intoxication (heart, brain, skeletal muscle, kidney). This differential expression is more accentuated in old mice, possibly pointing to the special relevance of these cytokines to organ homeostasis in old age. These findings may shed new light on the physiological functions of IL-1alpha and IL-1beta, and may also lead to the development of improved therapeutic approaches, based on the specific manipulation of these cytokines.

Full text

INTRODUCTION

IL-1 plays a cardinal role in organ homeostasis and defense, due to its remarkable capacity to modulate inflammatory, immune and metabolic responses [Reviewed in 1]. This prototypical, proinflammatory cytokine is highly pleiotropic and ubiquitously expressed. It is synthesized and secreted by diverse cells, including lymphoid and stromal cells, and affects nearly every cell type, often in concert with other cytokines or small protein mediators [1].

As a host-defense promoting factor, IL-1 stimulates T and B lymphocytes, potentiates the activity of NK cells and macrophages, and induces the generation of chemokines, cytokines and other inflammatory mediators [1]. It also strongly induces and potentiates delayed type hypersensitivity (DTH) responses. As a highly inflammatory cytokine, IL-1 may prove noxious to tissues when active in excess, as illustrated by its association with various pathological states, such as autoimmune diseases (type I diabetes, rheumatoid arthritis), atherosclerosis and sepsis, all mediated by excessive or disturbed inflammatory reactions [1].

The two agonist IL-1alpha and IL-1beta proteins and the IL-1 receptor antagonist (IL-1Ra) are the three most studied members of the IL-1 family [1]. They are encoded by distinct genes, which bind to the same IL-1 receptors (IL-1Rs). In their recombinant form, IL-1alpha and IL-1beta share the same spectrum of biological activities following binding to the IL-1Rs. However, some reports point

out that IL-1alpha and IL-1beta may nevertheless differ in their biological activities [2-5], a possibility which may prove more likely under physiological conditions and in the context of the producing cell. The possible existence of such a differential *in vivo* activity of IL-1alpha *versus* IL-1beta is, in addition, suggested by the distinct intracellular metabolism/processing of IL-1alpha and IL-1beta. Thus, IL-1alpha and IL-1beta differ in the subcellular compartments in which they are active; IL-1alpha is mainly active as a cytosolic precursor (31 kD) and a membrane-associated molecule (23 kD), being only marginally secreted (mainly by macrophages), while IL-1beta is regarded as active only in its secreted form. The importance of IL-1alpha and IL-1beta compartmentalization within the producing cell or its microenvironment, as a factor which determines their array of biological activities, was demonstrated by us in studies on the tumorigenicity patterns of experimental fibrosarcomas that were transfected with the active forms of IL-1alpha and IL-1beta [6-10]. Thus, IL-1alpha-transfected fibrosarcoma tumor cells, which express the cytokine on the cell membrane, lose their tumorigenicity by becoming more immunogenic; this leads to development of efficient T cell-mediated, anti-tumor immune responses and tumor regression [6-8]. Conversely, IL-1beta-secreting fibrosarcoma cells emerge more malignant, suggesting the involvement of malignancy-promoting mechanisms [9, 10]. The IL-1Ra, which is the major natural inhibitor of IL-1 action and is widely expressed in various organs, emerges as an important *in vivo* regulator of IL-1 action [1, 11-14], including, perhaps, the dominant expression of IL-1 activity in lymphoreticular organs, as we have previously shown [15], and the yet unsettled issue of differential IL-1alpha *versus* IL-1beta physiological functions.

We report here on different patterns of IL-1alpha *versus* IL-1beta organ expression in young and old mice under steady-state conditions, based on organ cytokine characterization by immunohistochemistry and ELISA. Based on the findings presented in this work, it is suggested that the physiological effects of IL-1alpha and IL-1beta may be distinct in the context of organ homeostasis/defense.

MATERIALS AND METHODS

Mice

C57BL/6 mice were bred under conventional conditions at the animal facilities of the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. Eight-to-ten week old female mice represented young mice, whereas 24-26 month-old female mice represented old mice.

Recombinant cytokines and anti-cytokine antibodies

Murine rIL-1alpha (recombinant IL-1alpha, specific activity: >= 1×10^7 U/mg), rIL-1beta (specific activity: >= 1×10^7 U/mg), hamster anti-mouse monoclonal antibodies to IL-1alpha and IL-1beta, rabbit anti-mouse polyclonal antibodies (hyperimmune serum) to IL-1alpha, rabbit anti-mouse biotinylated polyclonal antibodies to IL-1beta and donkey anti-rabbit peroxidase conjugated IgG were all purchased from Genzyme Diagnostics, Cambridge, MA, USA. Goat anti-rabbit and anti-rat mouse absorbed IgG were purchased from Vector Laboratories, Burlingame, CA.

Immunohistochemistry

The analyzed organs/tissues were fixed in formalin and embedded in paraffin. For immunohistochemical staining, organ sections (4 mum thick) were prepared, serially deparaffinized with xylene and then rehydrated with ethanol. After washing in phosphate-buffered saline (PBS), the sections were blocked for 1 hour and then incubated in Cas-Blocker solution (0.5% w/v casein powder and 0.1% w/v sodium azide in PBS, Sigma Chemical Company, St. Louis, MO). Thereafter, the solution was stored at 4° C, after its pH had been adjusted to 7.4. All incubations were performed in humidity chambers. The primary antibodies were then added in Cas-Blocker solution; for IL-1alpha, rabbit anti-mouse polyclonal antibodies to IL-1alpha (diluted 1:200) for 15 min at room temperature, while for IL-1beta, rabbit anti-mouse polyclonal antibodies to IL-1beta (diluted 1:100) for overnight incubation at 4° C. Following extensive washings with 0.2% PBS-Triton-X-100 (3 consecutive 15 min immersions), goat anti-rabbit biotinylated IgG (secondary antibody) was applied for 30 min (diluted 1:200 in Cas-Blocker solution). After 3 additional washings in the same solution, the endogenous peroxidase activity of sections was quenched with 9:1 methanol-3% H_2O_2 solution (slides immersed for 15 min). Thereafter, slides were washed in PBS-Triton-X-100 solution and reacted with the avidin-biotinylated horseraddish peroxidase complex (Elite/Vectastain ABC kit, Vector Laboratories) for 30 min. Subsequently, 0.1 M solution of 3-amino-9-ethylcarbazole (AEC, Zymed

Laboratories, San Francisco, CA, USA) and 3,3'-diamino-benzidine (DAB, Sigma) were added for IL-1alpha and IL-1beta, respectively. After stopping the dye reaction by washing in distilled water, the sections were counterstained with haematoxylin and mounted in Crystal mount (Biomeda Corp. Foster City, CA, USA) for AEC and Eukitt Mounting Medium (O. Kindler Gmb H&Co, Freiburg, Germany) for DAB.

Omission of the specific primary antibody or its preincubation with excessive recombinant IL-1alpha or IL-1beta resulted in abolition or significant diminution of the specific staining (data not shown for the latter). In addition, the polyclonal anti-IL-1alpha/IL-1beta antibodies, used in this work, were produced by Genzyme under similar conditions; as such, they may serve as reciprocal irrelevant antibody controls.

Preparation of organ-derived conditioned media (CM) and lysates (LYS)

CM and LYS were prepared from organs of C57BL/6 mice, as described [15]. Thus, for the preparation of CM, the organs were removed on ice under aseptic conditions, rinsed three times in cold PBS, weighed and subsequently rinsed three times in a large volume of cold, antibiotic-containing (100 U/ml penicillin and 100 mug/ml streptomycin) serum-free RPMI-1640 (complete RPMI medium). Whole organs were then incubated for 48 hours in complete RPMI medium at 37° C. CM were then harvested, centrifuged (3,000 rpm for 10 min), and sterilized by millipore filtration (0.45 mum, Corning Glass Works, Corning, NY, USA). Supernatants were then stored at - 20° C before assay. For the preparation of LYS, organs were excised and rinsed as previously mentioned. Organs were then cut into small pieces (approximately 3 mm^3) and subsequently homogenized on ice (0.1 g tissue/ml in complete RPMI medium), using a homogenizer (Arthur H. Thomas Co., Philadelphia, PA, USA). The collected homogenate was cleared of debris by centrifugation (3,000 rpm, 10 min), and LYS were sterilized, aliquoted and stored at - 20° C. To minimize the potential non physiological effects caused by the tissue-proteases released under these conditions, lysates were prepared from freshly harvested organs/tissues without additional incubation in cell-culture medium. Also, to neutralize the effects of tissue proteases in LYS and CM preparations, a mixture of protease/oxygenation inhibitors (phenylmethyl-sulfonyl fluoride (PMSF)-0.2 mM, aprotinin-10 mug/ml, leupeptine-10 mug/ml, pepstatin-10 mug/ml and dithio-threitol (DTT)-0.5 mM, all purchased from Sigma) was added during the preparation of organ samples. Standardization was

achieved by diluting CM/LYS from the different organs in proportion to tissue weight. The arbitrary value of 0.1 g tissue/ml was chosen.

Protein level measurements in CM and LYS provided an additional criterion for standardization of the different samples. This was performed using the Bio-Rad protein assay (Bio-Rad Laboratories, Munich, Germany). A mean value of 245 mug/ml, with negligible variation, was found in LYS from the different organs. Similarly, the average value of 90 mug/ml was found in CM.

IL-1alpha ELISA

Ninety-six-well plates were coated overnight with hamster anti-mouse monoclonal antibodies to IL-1alpha (primary capture antibody, Genzyme, 1 mug/ml, diluted in 0.01 M carbonate coating buffer). Thereafter, the plates were washed twice with PBS/Tween and blocked for 2 hours at 37° C with PBS plus 10% FCS (fetal calf serum). After 2 additional washings with PBS/Tween, samples and standards of rIL-1alpha were added to microplates for overnight incubation at 4° C, followed by 4 washings in PBS/Tween. Rabbit anti-mouse polyclonal antibodies to IL-1alpha (detection antibodies, Genzyme, diluted 1:1000 in PBS/10% FCS), were then added to the microplates. After 1 hour of incubation at 37° C, plates were washed six times and developed by adding peroxidase-conjugated affinipure donkey anti-rabbit IgG polyclonal antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) (diluted 1:10000 in PBS/10% FCS) and its substrate, O-phenylenediamine dihydrochloride (OPD) (Sigma) for 45 min at 37° C. Absorbance was scored at a wavelength of 410 nm using the Dynateck MRX 5000 ELISA Reader (Dynex Technologies, Chantilly, VA, USA). The amount of IL-1alpha in samples was extrapolated from a standard curve of rIL-1alpha ranging from 15 to 405 pg/ml.

IL-1beta ELISA

The ELISA analyses of this cytokine were performed based on the Duoset ELISA Development System for mouse IL-1beta Genzyme. In short, ninety-six-well microplates were coated with hamster anti-mouse monoclonal antibodies to IL-1beta (capture antibody), as performed with IL-1alpha. Subsequently, biotinylated rabbit anti-mouse polyclonal antibodies to IL-1beta (detection antibody) were applied, followed by streptavidin-containing detection reagent and tetramethylbenzidine (TMB) as a substrate solution. Extensive washings

between these stages were performed with wash buffer (PBS with 0.05% Tween). The amount of IL-1beta in samples was extrapolated from a standard curve of rIL-1beta, ranging from 5 to 1,000 pg/ml.

Statistical analysis of results

Each experiment was repeated at least 3-5 times with similar patterns of results. Thus, 3-5 separate tissue/organ preparations of the diverse experimental samples were used; each was obtained from a pool of 3-5 mice. ELISA analyses were performed in triplicate; triplicate values in individual experiments did not differ from the mean by > 20%. The results obtained from the various experiments were pooled; shown are the means ± standard deviation. The statistical differences between the means representing the two age groups for individual organs were determined by Student's t-test. One way analysis of differences between the means representing different organs in each age group was done by the Scheffe test. A value of $p < 0.05$ was considered as significant in both tests.

RESULTS

Differential expression of IL-1alpha versus IL-1beta in organ CM

IL-1alpha is mainly detected in CM of the spleen and liver, while IL-1beta is detected most preponderantly in CM of the heart and kidney, as evaluated by ELISA (Figure 1). This marked expression of IL-1alpha in the CM of lymphoreticular organs may be attributed to the excessive cell stress, inflicted by the whole organ culturing conditions; indeed, damaged cells can secrete IL-1alpha [16, 17]. This pattern of expression, which is already evident in young mice, is accentuated in old mice. Thus, the expression of IL-1alpha is significantly higher in the lungs, spleen and liver of old mice (63.93, 194.9, 485 pg/ml, respectively), as compared with young mice (8.31, 73,18, 103.3 pg/ml, respectively). The differences between the two age groups are highly significant ($p = 0.001$, 0.001, and < 0.001, respectively for the lungs, spleen and liver). Likewise, the relative enhanced expression of IL-1alpha in the lymphoreticular organs (with some exceptions demonstrated by the lungs in young mice and small intestine in old mice), as compared with the other group of organs, is remarkable. Thus, in the two age groups, the levels of IL-1alpha expression in the spleen and liver significantly exceed those found in the heart, skeletal muscle,

kidney and brain ($p < 0.05$). The small intestine (young mice) and the lungs (old mice) display similar relative patterns of IL-1alpha expression ($p < 0.05$), except when compared with the skeletal muscle and the heart, respectively ($p > 0.05$).

As compared to IL-1alpha, IL-1beta is more evident in the kidney, skeletal muscle and heart. In old mice, IL-1beta expression is more marked in the first two mentioned organs. Thus, IL-1beta levels of 383.7 and 256.6 pg/ml are demonstrated in the kidney and skeletal muscle of old mice *versus* 176.2 and 81.63 pg/ml in young mice, respectively ($p < 0.001$ for the two organs). When the cytokine levels are compared in the two groups of organs, IL-1beta is found in significant relative excess (compared to the lymphoreticular organs) in the kidney, skeletal muscle and heart of old mice ($p < 0.05$). It should be noted that IL-1beta is practically not detectable in the spleen and small intestine of old mice.

IL-1alpha is expressed at low levels in highly specialized organs of young and old mice

IL-1alpha is demonstrable at low, sometimes borderline levels in LYS obtained from the heart, brain and kidney of young and old mice, when analyzed by ELISA (Figure 2). A slight increase in IL-1alpha expression was detected in old age in these organs, but this was not statistically significant. Thus, the different mean levels of IL-1alpha in organs of old and young mice were as follows: 10.05 *versus* 5.264 pg/ml (heart, $p = 0.026$), 13.54 *versus* 9.35 pg/ml (brain, $p = 0.263$), 6.76 *versus* 4.07 pg/ml (kidney, $p = 0.113$), respectively for the heart, brain and kidney. Higher levels of IL-1alpha were detected in the skeletal muscle of old and young mice (31.44 and 35.17 pg/ml, respectively, $p = 0.417$). Nevertheless, the differences between these four organs were not statistical significant ($p > 0.05$). The levels of IL-1alpha expression in these organs of old and young mice at steady-state conditions, as detected by the specific immunohistochemical stainings, are, in general, low, as representatively shown in Figure 2 for the kidney (A) and heart (B). In the kidney, IL-1alpha is most clearly demonstrated in cortical tubular cells, while the staining for IL-1alpha in the heart is cytoplasmic and of a diffuse nature, with variable intensity in the different fibers.

In the brain, the specific staining for IL-1alpha is most clearly detected in the cytoplasm of cerebral cortex macrophage-like cells, possibly astrocytes and microglia cells (data not shown). The skeletal muscle is characterized by a

diffuse, uneven distribution of IL-1alpha in the myocytes, greatly resembling the pattern of IL-1alpha expression in the heart (data not shown). The intensity of staining in the latter organs is comparable with that shown for the kidney and heart.

Thus, IL-1alpha appears to be expressed at low levels in privileged organs of both young and old mice under steady-state conditions. This apparent stringent regulation of IL-1alpha expression is probably essential in these organs, as seemingly, it is not altered by aging.

IL-1alpha is expressed at high levels in lymphoreticular organs of old mice

An enhanced level of IL-1alpha expression in the lymphoreticular organs (lungs, spleen, small intestine, liver) of old mice was detected by the immunohistochemical stainings, when compared to the same organs of young mice and the second category of organs (representatively displayed for the lungs and small intestine in Figure 2C and Figure 2D, respectively). The basic pattern of the histological distribution of IL-1alpha, as demonstrated here, does not change with age, and predominantly involves the bronchiolar epithelial cells (lungs), lumen-facing villous epithelial cells (small intestine), hepatocytes of perivenular regions (liver) and cortical macrophages of the spleen (data not shown for the latter two organs). The relative increase in IL-1alpha-specific staining in the liver and spleen of old mice is of comparable intensity to that shown for the small intestine and lungs. Overall, a "lumen-oriented" pattern of expression is thus exhibited.

ELISA analyses of LYS obtained from the lymphoreticular organs demonstrate an equally elevated IL-1alpha expression in both age groups, with the exception of the lungs, where the level of IL-1alpha in old mice is considerably higher (220.5 *versus* 118.9 pg/ml respectively, $p = 0.002$) (Figure 2). The levels of IL-1alpha expression in all tested lymphoreticular organs significantly exceed those demonstrated in the second analyzed group of organs ($p < 0.05$). The only exception is the relative expression of IL-1alpha in the small intestine compared with the skeletal muscle, where the difference was not of statistical significance ($p > 0.05$). In addition, the levels of IL-1alpha in the spleen, liver (young and old mice) and lungs (young mice) appear similar ($p < 0.05$).

Despite the comparable ELISA-characterized findings obtained in young mice (relative to old mice), the demonstration of enhanced IL-1alpha expression in lymphoreticular organs of old mice by both immunohistochemistry and ELISA probably implies a different regulation of IL-1alpha-expression in these organs upon aging.

Generalized enhanced expression of intracellular IL-1beta in old mice

A distinct widespread elevation of intracellular IL-1beta expression was observed in both privileged and lymphoreticular organs of old mice, as assessed by immunohistochemistry, compared to young mice (Figure 3). The basic histological distribution of IL-1beta, as representatively displayed for the heart, kidney, lungs and small intestine, is similar in both age groups. As IL-1alpha, it was mostly detected in cortical tubular cells in the kidney (Figure 3A), while, in the heart, it was demonstrable as a diffuse myocardial staining (Figure 3B). In the brain (mainly cerebrum), and skeletal muscle, IL-1beta was detected in the cytoplasm and nuclei of neurons and macrophage-like cells (possibly astrocytes or microglia cells), and diffusely in myocytes, respectively (data not shown). IL-1beta expression in the lymphoreticular organs is characteristically exhibited in lumen-oriented cells: bronchiolar epithelial (lungs), villous epithelial (small intestine) (Figure 3C, Figure 3D, respectively) and perivenular hepatocytes (liver, data not shown). In the spleen, IL-1beta is mostly demonstrable in red pulp macrophages; a fainter staining is also present in lymphocytes of the white pulp (data not shown). The relative increase in IL-1beta-specific staining in the brain, skeletal muscle, liver and spleen of old mice is of comparable intensity as that shown for the organs in Figure 3. It should be noted, however, that despite the equivalent intensity of IL-1alpha and IL-1beta staining, IL-1beta is most probably present in tissues in lower amounts or alternatively, in a different configuration which may render it more difficult to detect upon formalin fixation. This may be inferred from the prolonged (overnight) incubation with the specific anti-IL-1beta antibody, needed to demonstrate this cytokine, as compared to the short (15 min) incubation for IL-1alpha.

With the exception of the lungs and spleen, in which the differences between IL-1beta levels in the two age groups were not statistically significant ($p = 0.145$, 0.110, respectively), relative enhanced levels of IL-1beta were also detected by the ELISA analyses in most organs of old mice, as compared to young mice (Figure 3). Thus, the following respective mean levels of IL-1beta were found: in

the heart, 93.37 *versus* 18.37 pg/ml (p = 0.001), skeletal muscle, 67.52 *versus* 15.24 pg/ml (p = 0.004), kidney, 78.47 *versus* 19.12 pg/ml (p = 0.002), brain, 124.2 *versus* 14.27 pg/ml (p = 0.000), small intestine, 78.92 *versus* 27.97 pg/ml (p = 0.002), and liver, 315.7 *versus* 156.8 pg/ml (p = 0.004). Notable also is the relatively "uniform" expression of this cytokine in the various organs of young and old mice, respectively (with the exception of the liver). In this respect, the differences between the mean IL-1beta values in the various analyzed organs were not statistically significant (p > 0.05) in the corresponding age groups. The discrepancy between the marked immunohistochemical staining for IL-1beta and the low levels of ELISA-detected-IL-1beta in the lungs and spleen may indicate differences in sensitivities of the methods or specific susceptibility of the IL-1beta molecules to the specified experimental conditions, resulting in alteration of antigenicity or even degradation.

Differential increase in the relative expression of organ IL-1alpha and IL-1beta upon aging

The old/young ratio, regarding IL-1alpha and IL-1beta organ expression in the two age groups, respectively, was addressed, aiming to highlight the effect of aging in this respect. The calculation of the ratio was based on the respective values relating to IL-1alpha and IL-1beta expression, as detected in the young and old mice by the ELISA analyses. As shown in Figure 4, for organ CM, aging appears to enhance the relative predominance of IL-1alpha in the spleen, liver and lung. On the other hand, analysis of organ LYS especially emphasizes the relative predominance of IL-1beta in the brain, heart, kidney and skeletal muscle. It thus appears that old age is characterized by an overall increase in organ IL-1 expression. Based on their patterns of organ expression, IL-1alpha may play a more dominant role in the lymphoreticular organs, while IL-1beta may prove more influential in the more specialized and vulnerable organs.

DISCUSSION

In this study, we have demonstrated different patterns of IL-1alpha and IL-1beta organ expression under steady-state conditions, in young and old mice, grown under conventional, non-germ-free conditions. Thus, IL-1alpha is predominantly expressed in lymphoreticular organs, while, IL-1beta is more evident in organs specialized in functions other than rebuffing invasion by infectious or toxic

agents. This strongly suggests that the physiological role of IL-1alpha may differ substantially from that of IL-1beta.

The experiments presented in this study were performed in C57BL/6 mice, which preferentially develop Th1 responses and DTH reactions. Similar IL-1 distribution was observed in young (8-10 wks) female BALB/c mice, which tend towards to Th2 responses and antibody production. The patterns of organ IL-1 expression in old BALB/c mice have still to be assessed.

IL-1alpha and IL-1beta differ in their compartmentalization within the producing cell and its microenvironment, as well as in distinct patterns of generation and secretion, as indicated above [1]. Consequently, the physiological roles of these two IL-1 molecules may differ. Thus, IL-1alpha, expressed at low levels as a membrane-associated molecule and apparently acting as a focused adjuvant, possibly activates immune and other cells in a juxtacrine manner *via* ligation of IL-1 receptors. Cytosolic IL-1alpha may have important intracrine and autocrine homeostatic functions in the producing cells, such as those related to cell growth, differentiation, senescence and apoptosis [1, 18-24]. In contrast, secreted IL-1beta which acts in an autocrine, paracrine and even endocrine manner, is viewed as a major mediator of inflammatory responses [1]. In this context, we have shown that regression of IL-1alpha-transfected fibrosarcoma tumor cells is largely mediated by cytotoxic T lymphocytes (CTLs), helper T-lymphocytes (Th1) cells and their secreted cytokines with some contribution from NK cells and macrophages. In contrast, the same fibrosarcoma cells engineered to express the secreted form of IL-1beta proved to be more tumorogenic than the parental cells [6-10]. We have suggested that membrane-associated IL-1alpha expressed on the tumor cells serves as a cell-associated adjuvant that facilitates their interaction with IL-1R-expressing T-lymphocytes and non-adaptive effector cells. Conversely, IL-1beta may induce metalloproteinase synthesis and neoangiogenesis, and thus enhance tumor growth [9, 10].

The marked expression of IL-1alpha in various types of cells of lymphoreticular organs, apparently highlights IL-1alpha as a major mediator of local immunity in these organs, possibly including delayed type cellular (DTH) responses, targeted against intracellularly-acting microorganisms. Lumen-facing epithelial cells and liver parenchymal cells may play an important role in this setting, as demonstrated here by the marked expression of IL-1alpha in

pulmonary/intestinal epithelial cells and hepatocytes, and by the capacity of these cells to generate important immunomodulatory factors, such as IL-1, TNF-alpha chemokines, colony-stimulating factors, prostaglandins and reactive oxygen species, in response to infectious/toxic stimulations [25-30].

In contrast, the expression of IL-1beta appears overall more characteristic of highly specialized and more vulnerable organs; as exemplified by the heart and brain, where even minor compromises in their function can gravely affect homeostasis. Thus, a differential homeostatic/defensive role of IL-1beta is indicated, suggesting that the *in vivo* effects of IL-1beta might be more rigorously regulated and thus, relatively devoid of tissue-damaging effects. Indeed, as compared to IL-1alpha, *in vitro* IL-1beta expression is more tightly controlled at the level of transcription, mRNA stability, translation, processing and secretion [1]. Also, through IL-1beta-induction of organ anti-inflammatory agents, such as IL-6 and the IL-1Ra, generation of adverse effects can be downregulated. In this context, it was shown that IL-1beta and IL-1Ra genes are co-regulated in response to inflammatory stimuli [31] and IL-6, which is more dominantly expressed in these organs [15], can inhibit the expression and action of IL-1 and TNF-alpha and stimulate the secretion of the IL-1Ra [32]. Moreover, IL-1beta can directly induce the expression of mediators of innate immunity, *e.g.* defensins, mannose receptors, complement components and NK cells [1, 33-37], characterized by a short-term and self-limited activity. Lastly, IL-1beta has been shown to mediate normal metabolic/electric functioning and tissue repair after ischemic or infectious insults in the heart and brain [38-40], and antagonize apoptosis [41-44]. Thus, in addition to defense functions, organ IL-1alpha and IL-1beta may have differential, not yet fully understood homeostatic roles beyond the realm of immunity. Differentially expressed organ IL-1Ra (shown quantitatively more dominant in lymphoreticular organs) [11, 12], in possible conjunction with altered expression of organ IL-1R [11], might distinctly affect the expression of IL-1alpha *versus* IL-1beta and thus, emerge as crucial in determining the fine-tuning of organ IL-1 expression.

An increase in the expression of both IL-1alpha and IL-1beta is observed in old mice, demonstrating cytokine-organ specificity as emphasized above. These alterations possibly delineate a non-selective response, which may compensate in an organ-specific manner for the more specifically defined deficits/defects which characterize the deteriorating immunity of old age [45, 46]. As it is shown

to be widely intracellularly elevated in organs of old mice, IL-1beta may emerge as more typical of senescence.

Most studies have related active inflammatory responses to IL-1beta secretion, while putting less emphasis on the comparative role of IL-1alpha in such processes. Also, IL-1beta cellular/organ expression has mostly been studied following stimulation by inflammatory inducers, rather than in non-stimulated cells or in subjects under steady-state, homeostatic conditions. The accepted association of IL-1beta with destructive inflammatory responses is not necessarily in contradiction with its "docile", perhaps inflammation-attenuating, defensive roles proposed here. *In vivo*, IL-1beta may induce differential and multi-faceted effects, proving, for example, cell-damaging and even cytocidal when exceeding critical quantitative thresholds or acting under drastic alterations of the microenvironment [47, 48]. The expression of IL-1alpha in later stages of the inflammatory response, as compared to IL-1beta, may further attest to the preferential role of IL-1alpha in delayed defense mechanisms [49, 50].

Overall, it is shown here that IL-1alpha and IL-1beta display different patterns of organ expression, most clearly evident in old age and involving sites considered as "non-immunological". These findings may indicate that these two IL-1 molecules may play distinct roles in the context of organ defense and homeostasis, without discarding the possible relevance of parallel and even complementing activities under specific circumstances. The "net" activity of IL-1 in a certain organ may thus depend on the type and amount of the IL-1 molecule, IL-1Rs and IL-1Ra expressed, as well as on the local cytokine network.

CONCLUSION

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Figures

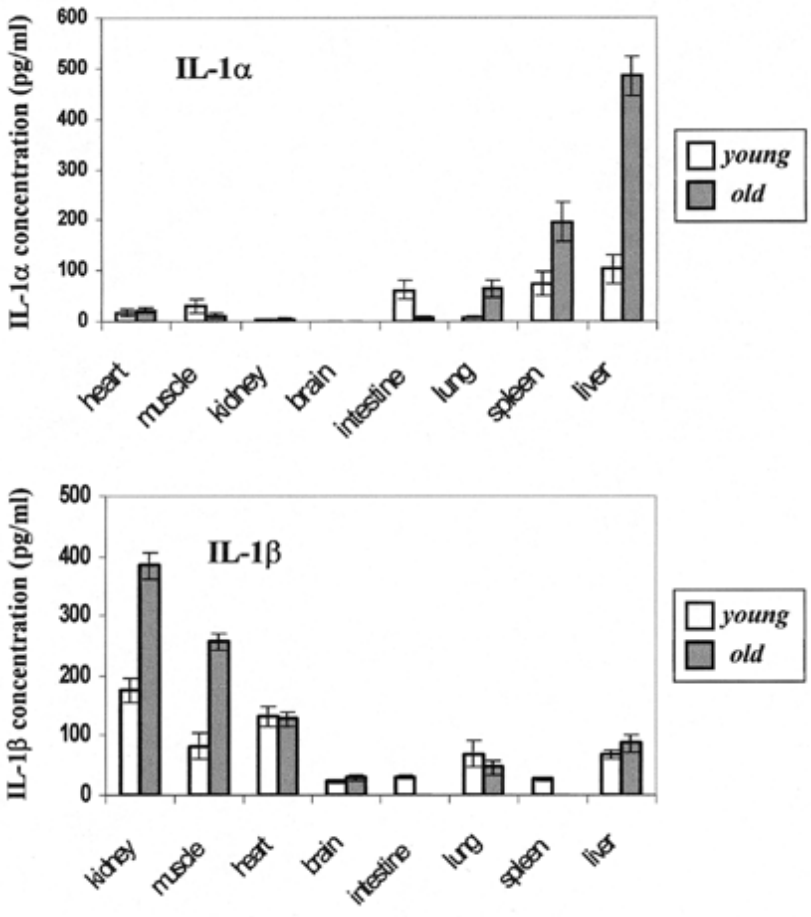


Figure 1

IL-1alpha *versus* IL-1beta expression in organ CM. The expression of IL-1alpha and IL-1beta in organ CM was assessed by ELISA in young (empty bars) and old mice (solid bars), as described in the materials and methods section, and is presented in pg/ml. The respective IL-1alpha and IL-1beta expression for young and old mice is comparatively depicted for each organ. To illustrate this better, the sequence of organs shown is arranged based on the decreasing expression of IL-1alpha or IL-1beta.

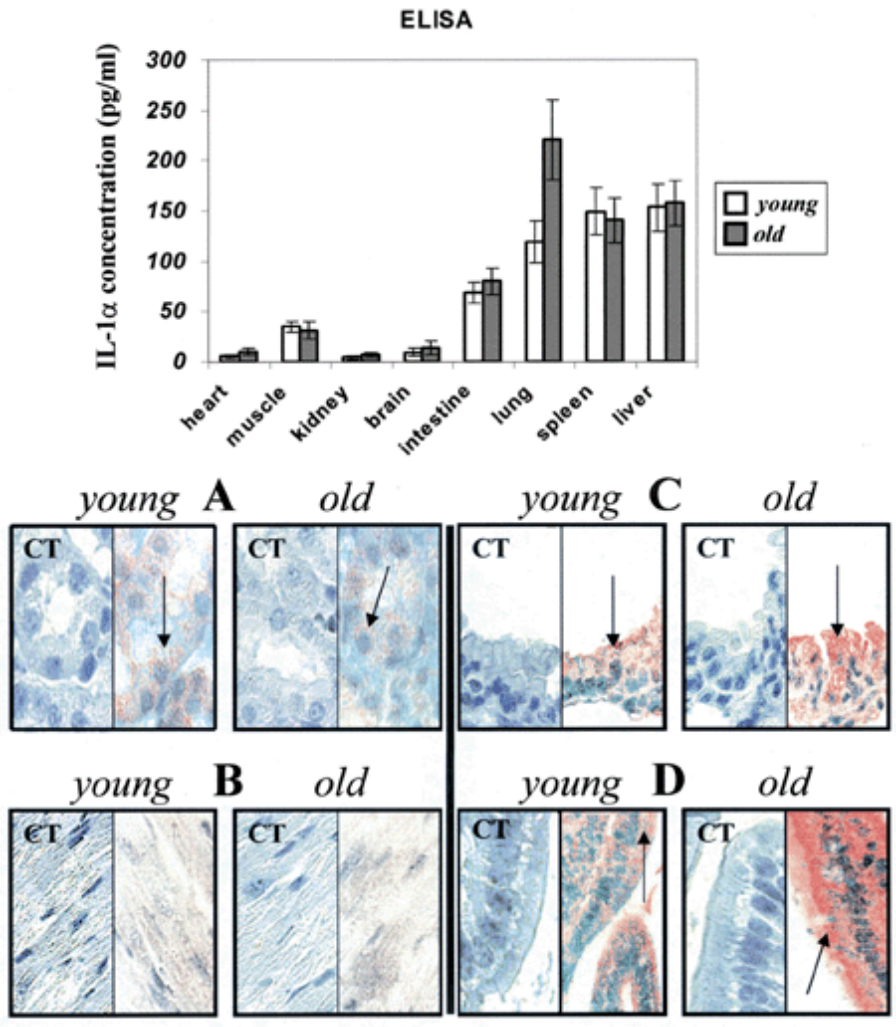


Figure 2

Organ intracellular expression of IL-1alpha in young *versus* old mice assessed by ELISA and immunohistochemistry. The presence of immunoreactive IL-1alpha in organ lysates was detected by commercial ELISA kits and is comparatively presented for young (empty bars) and old (solid bars) mice. IL-1alpha expression, manifested by the red staining by the AEC dye, was characterized in the kidney tubular cells (arrow, **A**), heart myocardium **(B)**, lung bronchiolar cells (arrow, **C**) and small intestine epithelial cells (arrow, D) of young and old mice by immunohistochemistry, using formalin-fixed, paraffin-embedded sections, as described in the materials and methods section. The specificity of IL-1alpha was determined using specific rabbit anti-mouse polyclonal antibodies to IL-1alpha. "CT" denotes control

histological sections stained in the absence of the primary, specific antibody. Magnification of all figures is x 800.

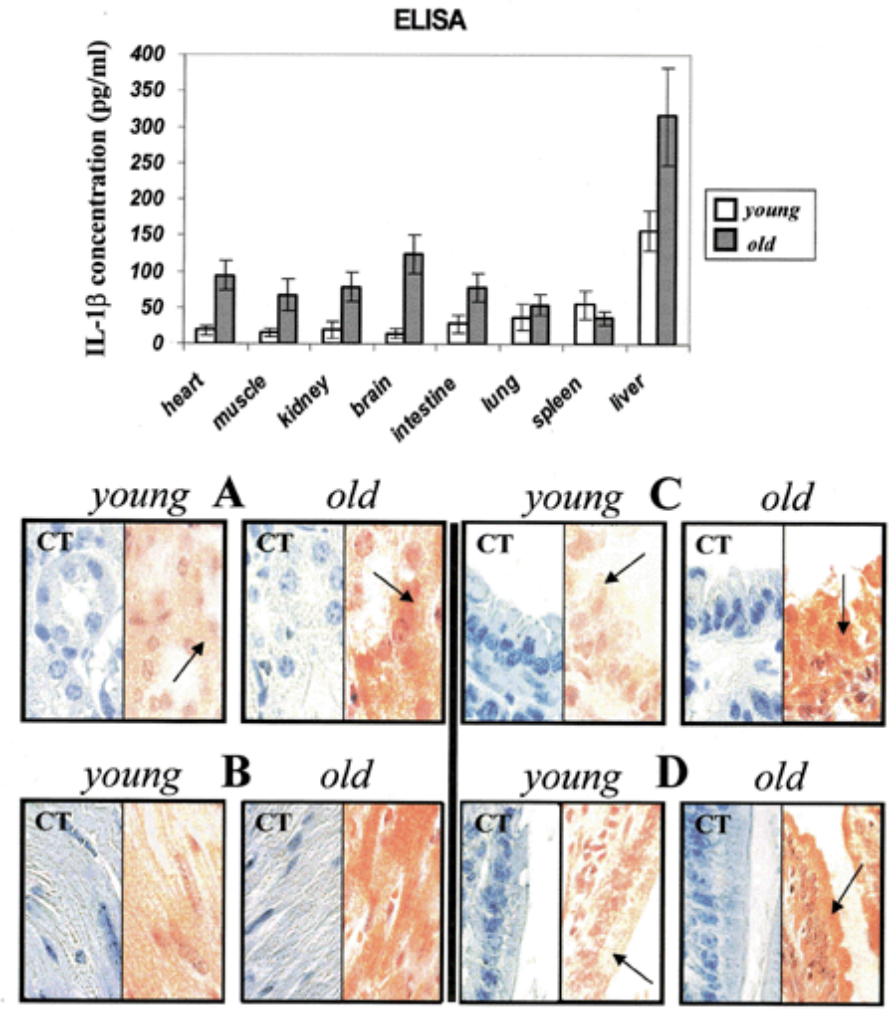


Figure 3
Organ intracellular expression of IL-1beta in young *versus* old mice assessed by ELISA and immunohistochemistry. The presence of immunoreactive IL-1beta in organ lysates was detected by commercial ELISA kits and is comparatively presented for young (empty bars) and old (solid bars) mice. IL-1beta expression, manifested by the brown-reddish staining by the DAB dye, was characterized in the kidney tubular cells (arrow, **A**), heart myocardium **(B)**, lung bronchiolar cells (arrow, **C**) and small intestine epithelial cells (arrow, **D**) of young and old mice by immunohistochemistry, using

formalin-fixed, paraffin-embedded sections, as described in the materials and methods section. The specificity of IL-1beta was determined using specific rabbit anti-mouse polyclonal antibodies to IL-1beta. "CT" denotes control histological sections stained in the absence of the primary, specific antibody. Magnification of all figures is x 800.

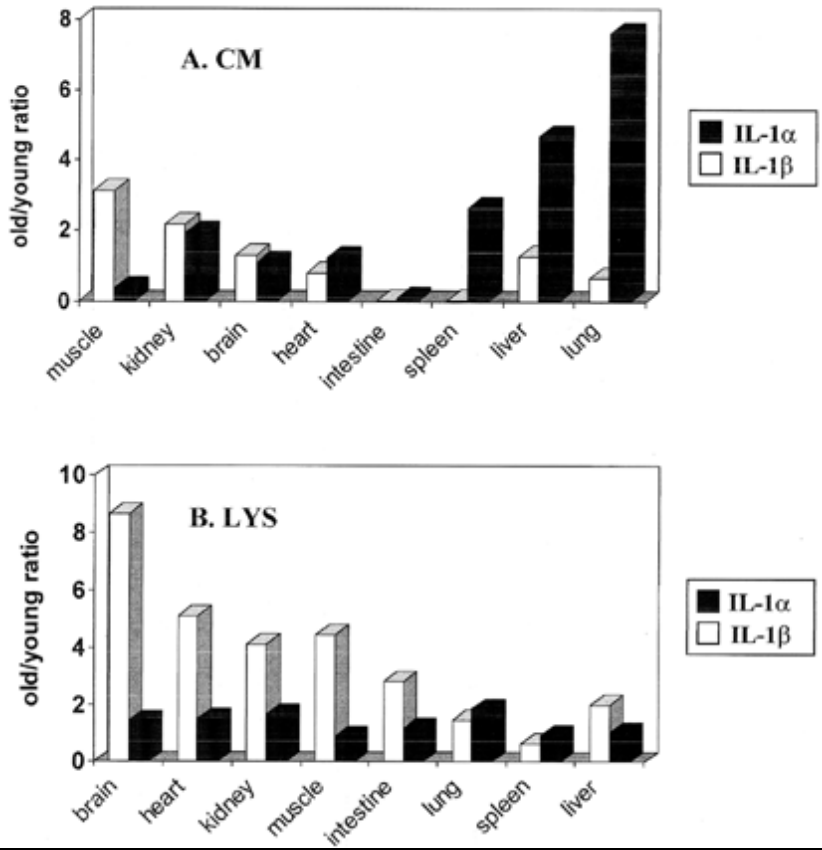


Figure 4

Relative organ expression of IL-1alpha and IL-1beta in old compared with young mice. The old/young ratio of organ cytokine expression was calculated, based on IL-1alpha and IL-1beta values, as detected in young and old mice by the ELISA. The respective values, obtained by ELISA for organ CM **(A)** and LYS **(B)** are presented comparatively for IL-1alpha (solid bars) and IL-1beta (empty bars). To illustrate this better, the sequence of organs shown it arranged based on the decreasing value of old/young ratio of IL-1alpha/IL-1beta expression.

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The effect of interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) on granulocyte macrophage-colony stimulating factor (GM-CSF) production by neuronal precursor cells

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IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

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