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A Defective Protein Kinase C Anchoring System Underlying Age-Associated Impairment in TNF-α Production in Rat Macrophages[1]

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The ability of macrophages to secrete cytokines is important in host responses to infections inflammatory stimuli, both of which are altered with aging. In this study, age-associated changes in the release of TNF-α from LPS-stimulated rat alveolar macrophages were determined and correlated with a decrease in the level of RACK1, the anchoring protein involved in protein kinase C translocation and activation. Macrophages from aged rats produced ~50% less TNF-α than those from young rats. This effect was observed independently from the concentration of LPS used and the time considered. The decrease observed was associated with a defective PKC translocation, due to a reduction in the expression of RACK1, whereas no differences were detected in the expression of LPS receptor (CD14) or total PKC isoforms (α and β_{II}) in old and young rats. Use of RACK1 antisense oligonucleotide reduced the ability of young macrophages to respond to LPS, further supporting the idea that a deficit in RACK1 contributes to the functional impairment in aged macrophages and that age-induced macrophage immunodeficiencies are associated with alteration in signal transduction pathways. *The Journal of Immunology,* **1999, 163: 3468–3473.**

Aging is associated with increased vulnerability to certain infections and neoplastic diseases (1, 2). Most of the cells of the immune system are indeed altered by immunosenescence. Regarding the functions of monocyte-macrophage, it has been shown that the aging process depresses chemotaxis and phagocytosis (3) and induces a deficient respiratory burst (4). Furthermore, aging is associated with a decline in the ability of these cells to present Ags and to produce cytokines (5–9). It is reasonable that age-related immunodeficiencies may result in part from an age-associated alteration in the functional capacity of macrophages. The causes underlying the age-associated decline of cell functions are not clear; however, a defective signal transduction machinery seems to be involved (10, 11).

TNF-α is a potent paracrine and endocrine mediator of inflammatory and immune functions. TNF-α plays a crucial role in the development of a protective response to bacterial (12) and viral pathogens (13, 14). TNF-α has been also implicated in the mediation of a number of diseases including septic shock syndrome (15), cachexia (16), and AIDS (17, 18) and in the pathogenesis of certain autoimmune diseases (19). The primary sources of TNF-α are activated monocytes and macrophages (20), but it is also produced by a number of additional cell types including mast cells (21), granulocytes (22), B and T cells (23, 24), keratinocytes (25), astrocytes (26), etc.

Several studies have demonstrated that specific inhibitors of protein kinase C (PKC)[3] can block the secretion of TNF-α from LPS-stimulated macrophages, suggesting that PKC activation plays a significant role in the inflammatory response (27- 29). PKC is a family of phospholipid-dependent serine-threonine kinases involved in the signal transduction of hormones, neurotransmitters, and cytokines (30). Molecular cloning and biochemical studies (31) have provided a basis for classifying the different PKC isoenzymes into calcium-dependent (α, β_I, $\beta_{II,}$ and γ) and calcium-independent (novel PKC-δ, -ϵ, -η, -Φ, and -μ; atypical PKC-ζ, -ι and -λ) species. The activation of PKC results in redistribution (translocation) of the enzyme from cytosolic to membrane compartments (31). Recently, a family of proteins that interact with PKC has been described (32). These receptors for activated C kinase (RACKs) are 30- to 36-kDa proteins located in various subcellular compartments. The RACKs interact with PKC only in the presence of kinase activators in a specific and saturable manner (32–34). RACK1, a 36-kDa protein cloned from rat brain, is the best characterized member of the RACK family (for a recent review, see Ref. 35). Based on experiments with various PKC isoforms and isoform-specific Abs, it has been demonstrated that RACK1 preferentially interacts in vivo with PKC-β as compared with other PKC isoforms. Studies in LPS-activated macrophages have indicated the activation of several PKC isoforms. In particular, Shinji et al. (36) demonstrated that LPS induced the selective translocation of PKC-β, Shapira et al. (37) using isoform-specific Abs identified PCK-ϵ as the major isoform involved; and Herrera-Velit et al. (38) demonstrated that LPS induced the selective translocation of PKC-ζ.

In this study, we compared the release of TNF-α from alveolar macrophages of young and old rats. The capacity of macrophages from old rats to secrete TNF-α was significantly less than that of macrophages from young rats, despite the fact that the ability of macrophages to express the LPS receptor was not impaired in old

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[3] Abbreviations used in this paper: PKC, protein kinase C; RACK, receptor for activated C kinase; AM, alveolar macrophages.

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rats. Owing to the reputed role of RACK1 in PKC-mediated events, we have examined whether the lack of TNF-α release after LPS stimulation in aged alveolar macrophages might correlate with changes in RACK1 protein concentrations. We demonstrated that the age-associated decline in macrophage functions reflects indeed an impaired PKC signal transduction pathway and in particular correlates with a defective PKC anchoring system.

Materials and Methods

Animals

The experiments were performed with young (3- to 6-month-old), middle-aged (9- to 12-month-old) and old (>19 month-old) male Sprague-Dawley rats (Charles River, Calco, Italy). All animal care procedures were in accordance with the local Animal Care Committee, and no weight loss or decease was observed after receipt of rats in our animal facility. Rats were housed 2–3 per cage over wood-chip bedding and were allowed food and water *ad libitum*. Before sacrifice rats were quarantined for 2 wk and were acclimatized to a 12-h light-dark cycle.

Chemicals

LPS from *Escherichia coli* serotype 0127:B12 was obtained from Sigma (St. Louis, MO). Recombinant murine TNF-α (specific activity, $\sim 4 \times 10^7$ U/mg) and rabbit anti-murine TNF-α were obtained from Genzyme (Cambridge, MA); Abs against RACK1 and murine CD14 were obtained from Transduction Laboratories (Affinity, Nottingham, U.K.) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively; those raised against PKC-α and PKC-βII have been previously characterized (39). Salts were purchased from Carlo Erba (Milan, Italy), and electrophoresis reagents were from Bio-Rad (Richmond, CA). All reagents were purchased at the highest purity available.

Cells

Alveolar macrophages (AM) were collected by lavaging the lungs as described previously (40). Recovery ranged from 10 to 15×10^6 cells/animal, of which >98% were macrophages as determined by the Giemsa stain. Once washed and resuspended to 10^6 viable AM/ml, AM were allowed to adhere to plastic plates in serum-free RPMI 1640 (Sigma) containing 2 mM L-glutamine, 0.1 mg/ml streptomycin, 100 IU/ml penicillin, and 50 ng/ml gentamicin (medium). After adherence for 1 h at 37°C in 5% CO_2, the plastic plates were washed once with warm medium to remove nonadherent cells. Cells were then exposed to medium with 10% FCS (Sigma) and incubated with or without LPS at the concentration and times indicated.

Oligonucleotide treatment

A 22-base-long antisense oligonucleotide (Primm, Milan, Italy) corresponding to nucleotides 87–109 in the rat RACK1 sequence (41) was utilized: 5′-TTGCTCGGTCATGGCGTCAGAT-3′ (anti-RACK1). As control, the sense complement oligonucleotide was used: 5′-ATCTGCAGCCATGACCGAGCAA-3′ (senseRACK1). Both oligonucleotides contained phosphorothioate linkages to limit degradation. The transfection procedure was adapted from that of Locati et al. (42). Briefly, 10^6 AM/ml after adherence were cultured in the presence or absence of oligonucleotides (5 μM) in medium for 4 h. A final concentration of 10% FCS was then added, and AM were kept in culture for additional 48 h. Oligonucleotide treatment and culture conditions were not cytotoxic for the cells as assessed by lactate dehydrogenase leakage (data not shown). For TNF-α release, after 48 h of incubation the monolayers were washed once with PBS, and then fresh medium supplemented with 10% FCS and LPS, 10 ng/ml, was added for 1 h. For Western blot analysis of RACK1, AM were washed once with PBS and scraped in PBS; after centrifugation, the pellet was lysed in homogenization buffer as described below.

Assay for TNF

TNF content was assayed by determining the cytotoxicity of TNF against sensitive L929 cells, as previously described (43). The results are expressed in picograms per milliliter or nanograms per milliliter. TNF concentration was calculated against a standard curve with known amounts of recombinant murine TNF.

Western blot analysis

For CD14, PKC isoforms, and RACK1, $\sim 10 \times 10^6$ AM obtained from rats of different ages were lysed in 100 μl of homogenization buffer (50 mM Tris, 150 mM NaCl, 5 mM EDTA (pH 7.5), 0.5% Triton X-100, 50 μM PMSF, 2 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 μg/ml leupeptin) and denatured in 100 μl Laemmli sample buffer (44) for 5 min at 100°C. The protein content of the cell lysate was measured using a commercial kit (Bio-Rad). For TNF-α released in conditioned medium, 4×10^6 AM, after adherence, were cultured in medium with LPS, 10 ng/ml, for 24 h. The conditioned medium was quantitatively precipitated by the deoxycholate/trichloroacetate procedure described by Racchi et al. (45) and resuspended in 20 μl of Laemmli sample buffer and denatured. The intracellular proteins (10 μg) were then electrophoresed into a 12% SDS-polyacrylamide gel under reducing conditions, whereas the extracellular proteins were electrophoresed into a 18% gel. After electrophoresis, the proteins were transferred to nitrocellulose membrane (Amersham, Little Chalfont, U.K.) in 20 mM Tris, 150 mM glycine, 20% methanol, 0.03% SDS. The different proteins were visualized with a CD14 antiserum diluted at 1:200, RACK1 at 1:2500, PKC-α at 1:1250, PCK-β_{II} at 1:5000, and TNF-α at 1:250 as the primary Abs and developed using enhanced chemiluminescence according to the manufacturer's instruction (CDP-Star, NEN, Boston, MA). Molecular weight references were obtained by running one lane with prestained standard (Amersham).

In vitro PKC-βII translocation assay

AM (5×10^6) obtained from young and old rats were preincubated in a water bath at 37°C for 30 min in medium with 10% FCS in 15 ml polypropylene tubes to acclimate them. Then, LPS, 100 ng/ml, was added; after 5 and 10 min, AM were recovered by centrifugation for 5 min at 1200 rpm at 4°C. The pellets were resuspended in 500 μl of homogenization buffer (see Western blot analysis) without Triton X-100 utilizing a Teflon/glass potter. Cytosolic fractions were separated by centrifugation at $100{,}000 \times g$ for 60 min. The pellets were resuspended in the same volume of homogenization buffer by sonication for 2×15 s; this constituted the membrane + cytoskeleton fractions.

Reverse transcriptase-polymerase chain reaction

For determination of TNF-α mRNA levels, 4×10^6 AM from young or old rats were preincubated overnight in medium with 10% FCS to avoid adherence induction of TNF-α gene expression. After this time, fresh medium with or without LPS, 100 ng/ml, was added for 2 h. Total RNA was isolated by guanidinium thiocyanate-phenol-chloroform extraction (46). RT-PCR reactions were performed as previously described (47). Commercially available PCR primers for TNF-α and β-actin were purchased from Clontech Laboratories (Palo Alto, CA) and contained the following sequences. Mouse TNF-α: sense, 5′-ATGAGCACAGAAAGCATGACCGC-3′; antisense, 5′-CCAAAGTAGACCTGCCCGGACTC-3′. Mouse β-actin: sense, 5′-GTGGGCCGCTCTAGGCACCAA-3′; antisense, 5′-CTCTTTGATGTCACGCACGATTTC-3′.

The amplified PCR products from cytokine RNA are 692 bp for TNF-α and 540 bp for β-actin. In preliminary experiments, RNA concentrations and PCR cycles were titrated to establish standard curves to document linearity and to permit semiquantitative analysis of signal strength (5 and 50 ng for β-actin and TNF-α, respectively). Gels were photographed with type 55 film (Polaroid, Cambridge, MA). The image of the PCR products was acquired with a Nikon CCD video camera module (Nikon, Melville, NY). The optical density of the bands was calculated, and the peak area of a given band was analyzed by means of the Image 1.47 program for digital image processing (Wayne Rasband, Research Service Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD).

Statistical analysis

All experiments were performed at least twice; representative results are shown. Statistical significance was determined by Student's *t* test or Dunnett's multiple comparison test as indicated, after ANOVA.

Results

Diminished TNF-α production from LPS-stimulated AM from old rats

AM from young (3- to 6-month) or old (>18-month) male rats were obtained by bronchoalveolar lavage. The population recovered, which consists of >98% macrophages from young and old rats, was tested for TNF-α production in response to increasing concentrations of LPS (0–100 ng/ml). Fig. 1 shows that AM from old rats produced at least 50% less TNF-α than did AM from young rats at all concentrations of LPS tested. AM produced on average 12.5 ± 1.5 vs 3.2 ± 0.6 (1 ng/ml of LPS), 17.6 ± 1.0 vs

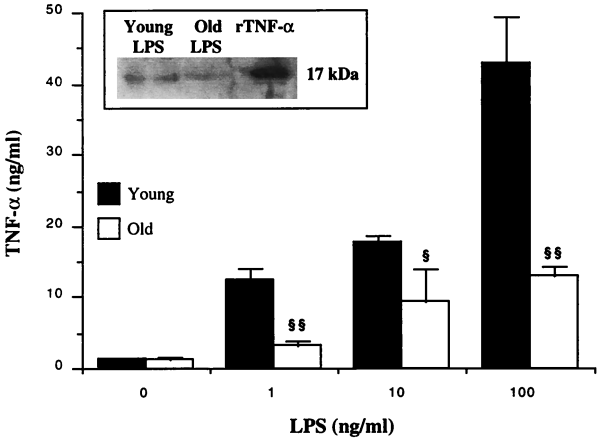

FIGURE 1. Diminished TNF-α production from old AM stimulated with LPS. AM were obtained from rats of different ages. AM (10^6/ml) were treated for 24 h with increasing concentrations of LPS (0–100 ng/ml). Each value represents the mean ± SD of 3–4 determinations. §, $p < 0.05$ and §§, $p < 0.01$ vs young AM, Dunnett's test. *Inset*, representative Western blot analysis of secreted TNF-α from AM treated for 24 h with LPS, 10 ng/ml. rTNF-α, recombinant murine TNF-α.

9.5 ± 4.5 (10 ng/ml of LPS), and 42.8 ± 6.4 vs 13.1 ± 1.3 (100 ng/ml of LPS) ng/ml of TNF-α from young vs old rats.

This effect was independent from the time of incubation. In time course experiments, the release of TNF-α from old AM was always less than the release from young AM when stimulated with LPS (Fig. 2).

This diminished capacity of AM from old rats to release TNF-α was confirmed by Western blot analysis of TNF-α immunoreactivity in conditioned medium from AM stimulated with LPS, 10 ng/ml (Fig. 1, *inset*). In the absence of LPS, no TNF-α release could be detected by Western blot analysis (data not shown). Furthermore, the defective TNF-α release from old AM was associated with a decrease in TNF-α mRNA expression as assessed by semiquantitative RT-PCR analysis (Fig. 3), suggesting a defect in the ability of AM to be activated. The quantification by densitometric analysis of the ratio of TNF-α to β-actin mRNA expression indicates a decrease of ~40% in TNF-α expression in AM from old rats stimulated with LPS (1.5 ± 0.3 vs 0.6 ± 0.3 of TNF-α/β-actin from young vs old rats).

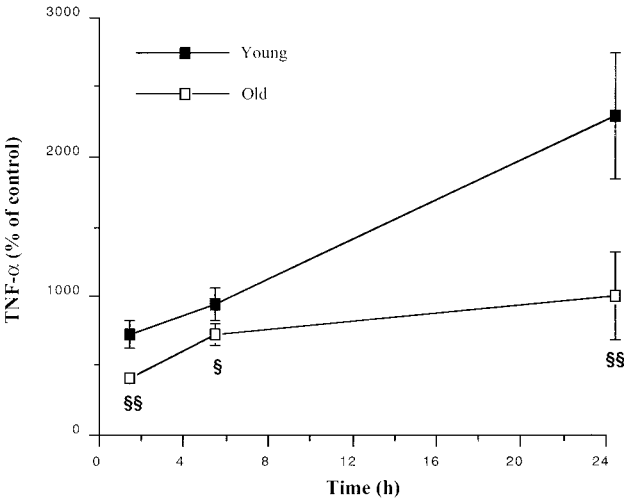

FIGURE 2. The decrease in TNF-α release in old AM was independent of the time considered. AM were obtained from rats of different ages. AM (10^6/ml) were treated for different times (1, 5, and 24 h) with 10 ng/ml LPS. Each value represents the mean ± SD of 3–4 determinations. §, $p < 0.05$ and §§, $p < 0.01$ vs young AM, Dunnett's test.

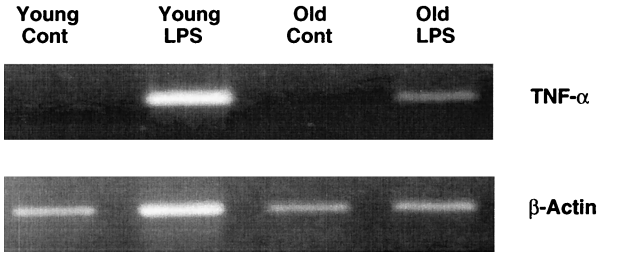

FIGURE 3. Effects of aging on TNF-α expression. RT-PCR analysis of LPS-induced TNF-α mRNA. AM were obtained from rats of different ages and treated as described in *Materials and Methods*. RT-PCR analysis of LPS-induced TNF-α mRNA.

Taken together, these data clearly indicate that AM from old rats produce significantly less TNF-α than AM from young rats.

Comparable amounts of CD14 and PKC isoforms, in the presence of a defective PCK translocation in old rats

We then explored the possibility that this diminished capacity of AM from old rats to produce TNF-α might be due to decreased levels of LPS receptor (CD14) or from a defect in the activation of AM. To test the first hypothesis, we compared CD14 expression in cell homogenates from young, middle-aged, and old rats by Western blot analysis of CD14 immunoreactivity. As shown in Fig. 4 a comparable or slightly higher CD14 expression was observed in old vs young AM. No evident difference in CD14 expression was observed in AM from middle-aged rats.

To test the second hypothesis, we compared the expression in cell homogenates obtained from AM of young, middle-aged, and old rats of two PKC isoforms (α and β_{II}) by Western blot analysis.

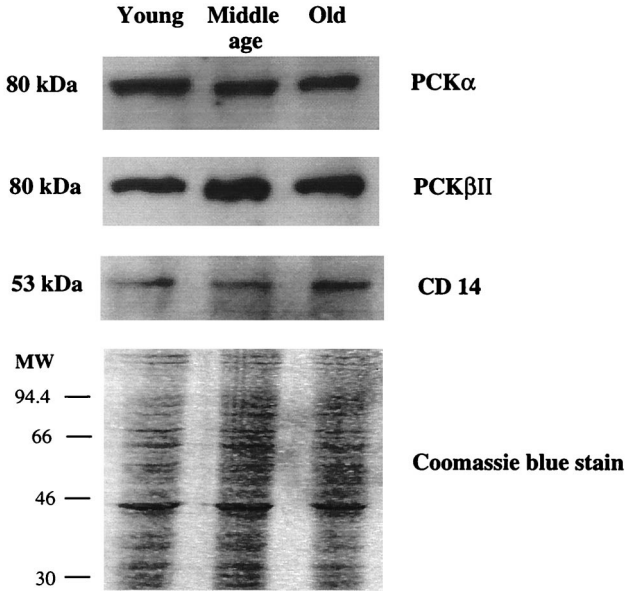

FIGURE 4. AM from old rats express comparable amounts of CD14, PKC-α, and PKC-β_{II}. AM were obtained from rats of different ages and treated as described in *Materials and Methods*. Representative Western blot analysis of CD14, PKC-α, and PKC-β_{II} immunoreactivity in cell homogenates from AM of rats of different ages. Protein was loaded at 10 μg for CD14 and 15 μg for PKC-α and PKC-β_{II}. For Coomassie blue stain, 10 μg of protein were loaded.

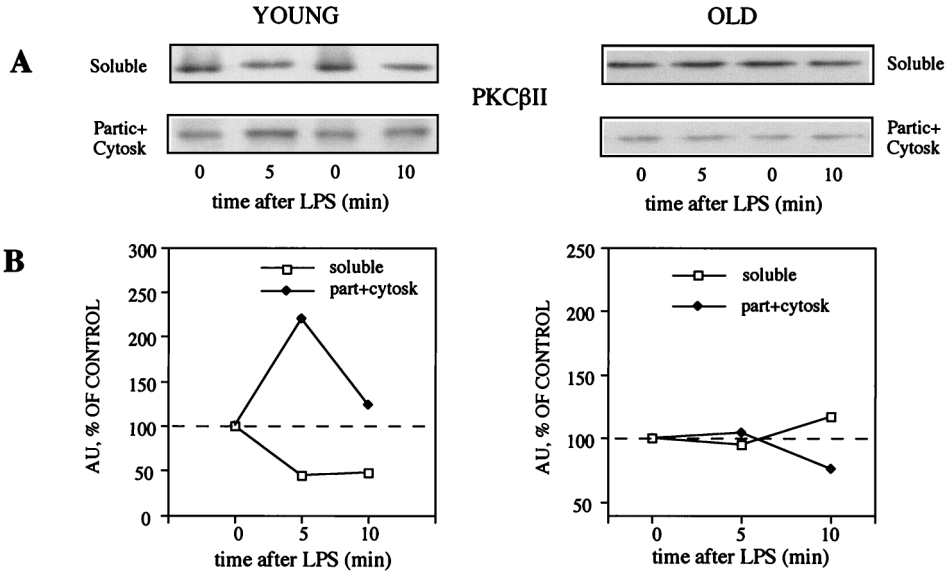

FIGURE 5. *A*, Representative Western blot analyses of PKC-βII immunoreactivities in young and aged AM after LPS stimulus. Proteins from soluble (20-μg) and membrane (10-μg) fractions were loaded and electrophoresed on the gels (12% SDS-PAGE). *B*, Quantification by densitometric analysis of PKC-βII immunoreactivities in soluble and membrane fractions after LPS; values are expressed in arbitrary units (AU) of optical density as percentage of control values (time 0, unstimulated AM). Cytosk, cytoskeleton fraction; part or Partic, particulate fraction.

As shown in Fig. 4, aging was not associated with changes in PKC isoform expression. Coomassie blue staining of gel reveals that equivalent amounts of proteins have been loaded onto gel (Fig. 4). To further investigate a possible defect in the signal transduction machinery, experiments of PKC translocation were performed. As shown in Fig. 5, LPS stimulation induced the translocation of PC-β_{II} from the cytosol to the particulate fraction in young AM, whereas under the same experimental conditions no translocation of PKC-β_{II} was observed in old AM.

Thus, the decrease in TNF-α production in old AM was not caused by a decline in CD14 or total PKC expression, but it was correlated with a defective PKC-β_{II} translocation.

A deficit in RACK1 contributes to the functional impairment in aged AM

RACKs are emerging as important proteins that bind activated PKCs and anchor them to specific intracellular sites where target substrates can be phosphorylated. As such, RACKs may serve to control PKC-related functions. RACK1 has been reported to bind with high affinity PKC-β_{II}, which selectively translocated in response to LPS in macrophages.

Fig. 6 inset depicts a representative Western blot of RACK1 immunoreactivity in cell homogenates of AM from rats of different ages. The Ab recognizes an immunoreactive species at ~36 kDa. An age-related decline in RACK1 expression is evident, which parallels a decline in the ability of these cells to release TNF-α in response to LPS (Fig. 5). The densitometric analysis showed a decrease of 48 $\pm$ 2% and 51 $\pm$ 1% in RACK1 expression in middle-aged and old rats vs young rats ($p < 0.01$).

To further confirm the involvement of RACK1 in LPS-induced TNF-α production, AM from young rats were treated with RACK1 antisense oligonucleotide to decrease RACK1 expression, and then TNF-α release was measured after stimulation with LPS. As shown in Fig. 7, *inset*, under our experimental condition, a ~30% (by densitometric analysis) decrease in RACK1 expression was obtained, which was associated with a significant decrease (~40%) in TNF-α release after 1 h of treatment: 183 $\pm$ 14 vs 312 $\pm$ 43 pg/ml TNF-α from antisense vs LPS-treated AM ($p < 0.01$). Control sense oligonucleotide did not affect RACK1 expression or TNF-α release (Fig. 7).

Taken together, these data demonstrate, for the first time, that aging in AM is associated to a defective PKC anchoring system and that RACK1 plays an important role in LPS-induced TNF-α release in AM.

Discussion

Aging is associated with alteration of immune functions, which has been correlated with the incidence of increased cancer and infections in elderly people (1, 2). Age-related immunodeficiencies may result in part from an age-associated alteration in the functional capacity of macrophages, which are an important accessory and effector cell population. It has been shown that the aging process depresses macrophage chemotaxis and phagocytosis (3), induces a deficient respiratory burst (4), and declines in the ability of these cells to present Ags and to produce cytokines (5–9). The reasons behind the decline of these functions are not fully understood, and impairment in signal transduction events have been implicated. The main purpose of this work was to characterize in AM the

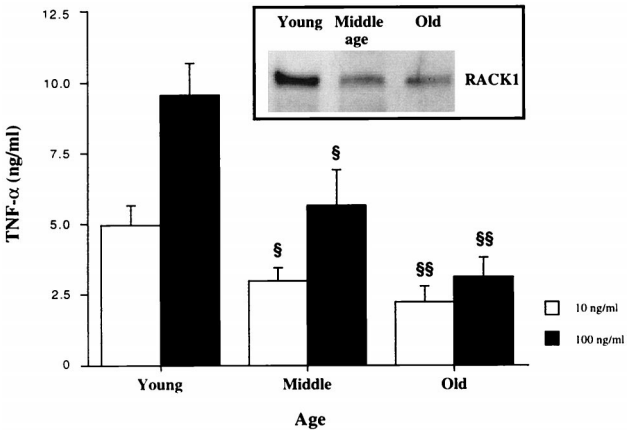

FIGURE 6. Age-related alteration in RACK1 expression and TNF-α release in AM. AM were obtained from rats of different ages and treated as described in *Materials and Methods*. AM (10^6/ml) were treated with 10 and 100 ng/ml LPS, and 24 h later TNF-α release was assessed. Each value represents the mean $\pm$ SD of 3–4 determinations. §, $p < 0.05$ and §§, $p < 0.01$ vs young AM, Dunnett's test. *Inset*, a representative Western blot analysis of RACK1 immunoreactivity in cell homogenate from AM of rats of different ages. Protein was loaded at 5 μg.

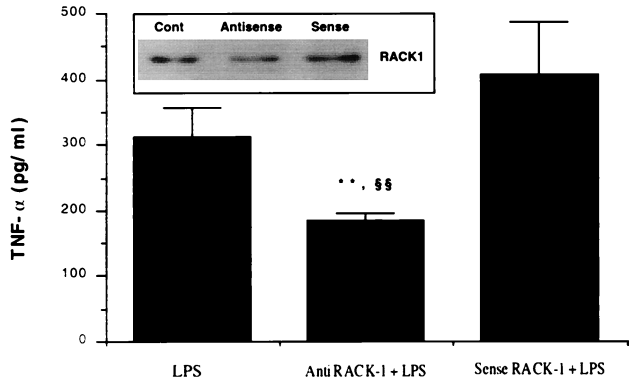

FIGURE 7. Antisense oligonucleotide modulates TNF-α release induced by LPS in young AM. AM were obtained from young AM and were treated as described in *Materials and Methods*. Each value represents the mean ± SD of 3–4 determinations. Statistical analysis was performed with Student's *t* test, with $p < 0.01$ vs LPS (§§) and $p < 0.01$ (**) vs sense RACK1 + LPS. *Inset,* a representative Western blot analysis of RACK1 immunoreactivity in cell homogenates of AM treated with medium alone, RACK1 antisense oligonucleotide (5 μM) or RACK1 sense oligonucleotide (5 μM). Protein was loaded at 7 μg.

age-associated changes in the release of TNF-α after LPS stimulation and to reveal the molecular mechanism(s) underlying immune senescence. It has been demonstrated (11) that aged monocytes display a decrease in IL-6 and TNF-α secretion, deficient PKC translocation (α, βI, and βII), and mitogen-activated protein kinase activation processes, and decreased expression of c-Fos and c-Jun. Our results demonstrate, for the first time, a decrease with age in the PKC-anchoring protein RACK1 expression in rat alveolar macrophages in spite of no change in levels of PKC-β_{II} or PCK-α, confirming that age-associated decline in macrophage functions reflects a defective PKC signal transduction pathway, specifically affecting the ability of PKC to translocate to the physiological anchoring sites. Furthermore, we demonstrated using antisense oligonucleotide in young macrophages that RACK1 plays indeed an important role in LPS-induced TNF-α release.

We found that aged macrophages, when compared with young macrophages, produced less TNF-α, independently from the concentration of LPS used or the time considered. This decrease was associated with a decrease in the mRNA expression of TNF-α, as assessed by RT-PCR analysis.

LPS is a potent activator of monocytes/macrophages. The major cell surface receptor for LPS on monocytes/macrophages is CD14. The LPS-deficient production of TNF-α observed in aged AM is not the results of a deficiency in the CD14 expression, as demonstrated by a comparable expression of CD14 in cell homogenates in old vs young AM.

We then investigated the signal transduction elements of aged cells, and, in particular, we focused our attention on PKC, which plays an essential role in LPS-induced TNF-α production (27–29). We demonstrated that RACK1 expression was deficient in aged alveolar macrophages in the presence of normal PCK isoform expression. RACK1 immunoreactivity was decreased in old macrophages by roughly 50% when compared with that in young macrophages. No changes were observed in the immunoreactivity of PKC-α and PKC-β_{II}, despite the alteration in RACK1 levels. The RACK1 immunoreactivity and TNF-α release show a progressive decrease with aging. RACK1 is essential in PCK anchoring to specific intracellular membranes where target substrates can be phosphorylated. As such, RACKs serve to control PKC-related functions We speculate that, because of the decreased RACK1 level, PKC does not bind to the membrane fraction in aged macrophages on LPS stimulation, resulting in a defective TNF-α production. These speculations are justified by the defective translocation of PKC-β_{II} that we observed in our system and are further supported by the literature, where a reduction in PKC translocation has been described in different immune cells (10, 11) and in particular, the data of Delpedro et al. (11), which demonstrated a defective translocation of PKC-α, PCK-β_{I}, and PCK-β_{II} isoforms in human monocytes.

The decrease observed in RACK1 expression in macrophages or the described deficiencies in PKC activation (10, 11) are probably not limited to the cells of the immune system. Indeed, it has been recently demonstrated a similar alteration in rat brain (48), where a defective PKC signal transduction system in the cortex of aged rats was described. In particular, this deficit was associated with a reduction in the levels of RACK1 anchoring protein and in PKC translocation (49), rather than changes in specific PKC isoform levels (50).

Macrophages are considered surveillance cells of the immune system. They are the first cells to encounter foreign elements (i.e., bacteria, virus, parasites, particles, etc.). Any deficiency in macrophage function will damage the individual. Understanding the age-associated immune function decline is of primary importance better to protect elderly people from infections and cancer. Here, we demonstrated that one of the causes of the deficiency in signal transduction observed in aged macrophages is a decrease in RACK1 expression, essential for PKC functions. At present, however, it is not clear what causes this decrease. A hormonal change in the cell environment might be responsible for the loss of RACK1. To our knowledge, however, the responsive elements in the promoter region of RACK1 have not yet been identified. This will help us better to understand the molecular mechanism(s) involved in immunosenescence and will open the way to exploration of the possibility of repairing age-associated immunological decline.

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