Age-associated impairment in TNF-α cardioprotection from myocardial infarction

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Cai, Dongqing, Munira Xaymardan, Jacquelyne M. Holm, Jingang Zheng, Jorge R. Kizer, and Jay M. Edelberg. Age-associated impairment in TNF-α cardioprotection from myocardial infarction. Am J Physiol Heart Circ Physiol 285: H463–H469, 2003. First published May 8, 2003; 10.1152/ajpheart.00144.2003.—Age-associated dysfunction in cardiac microvascular endothelial cells with impaired induction of cardioprotective platelet-derived growth factor (PDGF)-dependent pathways suggests that alterations in critical vascular receptor(s) may contribute to the increased severity of cardiovascular pathology in older persons. In vivo phage-display peptide library biopanning revealed a senescent decrease in cardiac microvascular binding of phage epitopes homologous to tumor necrosis factor-α (TNF-α), suggesting that its receptor(s) may be downregulated in older cardiac endothelial cells. Immunostaining demonstrated that TNF-α receptor 1 (TNF-R1) density was significantly lower in the subendocardial endothelium of the aging murine heart. Functional studies confirmed the senescent dysregulation of TNF-α receptor pathways, demonstrating that TNF-α induced PDGF-B expression in cardiac microvascular endothelial cells of 4-mo-old, but not 24-mo-old, rats. Moreover, TNF-α mediated cardiotropic pathways were impaired in the aging heart. In young rat hearts, injection of TNF-α significantly reduced the extent of myocardial injury after coronary ligation; TNF-α, 7.9 ± 1.9% left ventricular injury (n = 4) versus PBS, 16.2 ± 7.9% (n = 10; P < 0.05). The addition of PDGF-AB did not augment the cardioprotective action of TNF-α. In myocardial infarctions of older hearts, however, TNF-α induced significant postcoronary occlusion mortality (TNF-α 80% vs. PBS 0%; n = 10 each, P < 0.05) that was reversed by the coadministration of PDGF-AB. Overall, these studies demonstrate that aging-associated alterations in TNF-α receptor cardiac microvascular pathways may contribute to the increased cardiovascular pathology of the aging heart. Strategies targeted at restoring TNF-α receptor-mediated expression of PDGF-B may improve cardiac microvascular function and provide novel approaches for treatment and possible prevention of cardiovascular disease in older individuals.

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biopanning with a cyclic peptide pSKAN phagemid library (6 amino acid variable; ~10^{12} total complexity, Mo Bio Tech). Young adult (3 mo old) and aging (18 mo old) C57B61/L mice were anesthetized with 0.015 ml/g Avertin and injected with phage peptide library phage (10^{12} colony forming units (CFU)/200 μl PBS) via the tail veins. Four minutes after injection, the mice were euthanized, the hearts explanted, and the phage recovered with WK6amutS Escherichia coli. Age-specific phage pools were amplified and titrated for two additional rounds of biopanning enrichment. The phagemid DNA of the resultant clones were sequenced and translated amino acid motifs determined employing read through of all codons, as previously described for phage display library analysis (6, 13). Translated motifs were analyzed for homology to known cytokines (FASTA3), as determined by the first homologous mammalian sequence identified with E value <1. In addition, to probe the structural relevance of the phage motif cytokine binding epitopes, the regions of homology were mapped in the tertiary models and labeled by Cn3D3.0 software.

Individual phage clone in vivo cardiac vascular biopanning. To confirm the age-associated differential cardiac binding capacity of a candidate phage with homology to TNF-α, phage clone ΨY12, as well as helper phage without the insert, were prepared with TG1 E. coli to generation of phage motifs with supE suppression, as previously described (6). The ΨY12 phage (10^{12} CFU in 200 μl PBS) was injected into both 3- and 18-mo-old mice, as described above (n = 3, each group). The phages were recovered from the explanted hearts, as described above, with WK6amutS E. coli, which were then quantified by serial dilution titration.

In situ cardiac TNF-α receptor analysis. On the basis of the homology of ΨY12 to TNF-α, the potential age-associated changes in TNF-α receptor patterns were analyzed in situ. Cardiac sections of 3- and 18-mo-old wild-type mice were probed with goat antibodies directed against TNF-receptors 1 and 2 (TNF-R1 and -R2; Santa Cruz, Sc1079 and 1074) and developed with an anti-goat ABC kit with diaminobenzidine (Vector). Immunostained vascular density was quantified in the subendocardial tissue, as previously described: 16 high-power fields magnified ×40 per heart (n = 3, each group) (10). Counts were performed by two investigators in a blinded fashion.

TNF-α induction of PDGF-B in CMECs. The potential age-associated impairment in TNF-α-mediated induction of cardiac endothelial PDGF-B expression was measured in vitro. CMECs of 4- and 24-mo-old F344 rats were isolated and cultured, as previously described (10), with minor modifications. Briefly, the hearts were removed and minced in cardiac endothelial PDGF-B expression was measured in vitro. CMECs of 4- and 24-mo-old F344 rats were isolated and cultured, as previously described (10), with minor modifications. Briefly, the hearts were removed and minced in 24-mo-old F344 rats were isolated and cultured, as previously described (10), with minor modifications. Briefly, the hearts were removed and minced in DMEM with 2% FBS (30 ng/ml) for 3 h. Total DNA was then isolated and RT-PCR performed, as previously described (10). The primers for PCR were listed as follows: rat PDGF-B (sense) 5′-GATCCGCTCCTTTGATGATC-3′; rat PDGF-B (antisense) 5′-GTCTCACACTTGGATCCAG-3′; rat β-actin (sense) 5′-ATGGCAATGAGGGTCTCCGC-3′; and rat β-actin (antisense) 5′-TCCTTGCTTGTGATGCA-3′.

Age-dependent in vivo response to TNF-α. To probe the potential age-dependent effects of TNF-α on PDGF-B expression and protection from myocardial injury, sets of 4- and 24-mo-old F344 rats received intramyocardial injections of the growth factor, as previously described (10). Briefly, the rats were anesthetized with xylazine (5–10 mg/kg ip) and ketamine (80–90 mg/kg ip) and underwent a left intercostal thoracotomy. After the left anterior descending coronary artery (LAD) was identified, 100 ng of TNF-α in 50 μl PBS or PBS alone were injected through a 30 gauge needle, using a 250-μl Hamilton syringe. Two injections (25 μl injection 2 mm apart) were made at the middle left ventricular anterior wall. The chest wall was then closed, the lungs were inflated, the rat was extubated, and the tracheotomy was closed.

Age-dependent TNF-α-mediated induction of PDGF-B. Rats receiving pretreatments alone (TNF-α or PBS control, 4- and 24-mo-old, n = 3 each group) were euthanized 24 h postinjection. The hearts were excised, fixed, sectioned, and immunostained for PDGF-B (murine anti-PDGF-B, 376M, BioGenex) and visualized with a Texas red conjugated donkey anti-mouse antibody (Sc2785, Santa Cruz). PDGF-B staining was quantified in the subendocardial tissue in the anterior left ventricular wall at the level of the midpapillary muscles from each heart by identifying all PDGF-B-stained luminal structures, as previously described (10) (8 high-power fields magnified ×40 per heart). Two investigators performed quantification independently in a blinded fashion.

Age-specific TNF-α-mediated protection from myocardial infarction. The potential cardioprotective effects of TNF-α pretreatments were studied in a myocardial infarction model. One day after TNF-α (4 mo old, n = 4; 24 mo old, n = 10) or control (4 mo old, n = 4; 24 mo old, n = 10) intramyocardial injections, the rats were anesthetized, the heart was exposed, and the LAD was ligated just below (4 mo old) or 2 mm below (24 mo old) the left atrial appendage with 8-0 nylon sutures. Pallor and regional wall motion abnormality of the left ventricle confirmed occlusion. The chest wall was closed, and, after recovery, the rats were returned to the animal facility and kept for 14 days. The rats were killed and...
the hearts were harvested, fixed, and sectioned. Myocardial infarction size measured at the level of the midpapillary heart muscles was scored by Masson’s trichrome staining (20, 30), and the images were analyzed in a blinded fashion employing NIH Image Software version J1.22 (27, 38). Infarction size was expressed as a percentage of the total left ventricle myocardial area. Cardiac samples from aging rats that expired within 72 h of TNF-α injection were sectioned and analyzed for apoptotic induction by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining (in situ cell death kit no. 684817, Roche) (Fig. 3A). To measure the potential interactions of TNF-α and PDGF-AB, sets of rats were treated with a combination of TNF-α (100 ng/heart) and PDGF-AB (100 ng/heart) in 4-mo-old rats (n = 5) and 24-mo-old rats (n = 5), as well as by PDGF-AB alone (100 ng/heart) in 4-mo-old rats (n = 8) and 24-mo-old rats (n = 10).

Survival assay of TNF-α-treated old hearts. To investigate TNF-α-induced mortality after LAD occlusion in the 24-mo-old rats, additional sets of older rats received intramyocardial injection of TNF-α (100 ng/heart; n = 5) without subsequent coronary occlusion. These TNF-α-treated old rats were observed for 2 wk, and the survival number was recorded.

Statistical analysis. Comparisons of categorical variables were conducted using the binomial distribution or Fisher’s exact test, as appropriate. For nonnormally distributed continuous variables, a Wilcoxon rank-sum test was used. A two-tailed P value < 0.05 was considered statistically significant.

RESULTS

In vivo cardiac biopanning revealed repetitive homologies to TNF-α in the younger but not older cardiac-homing phage clones (2/101 vs. 0/100, P < 0.05) in Fig. 1A and B. In vivo injection with individual phage colonies confirmed the diminished cardiac homing of VY12 in the old murine heart (Fig. 1C), suggesting that the binding sites for the TNF-α-like phage motif may be decreased in the aging cardiac microvasculature. Immunostaining of 3- and 18-mo-old hearts revealed age-associated changes in one of the TNF receptors. TNF-R2 patterning was similar in the microvasculature throughout the young and old murine hearts (Fig. 2, A and B). Similarly, TNF-R1 was present in the microvasculature throughout the younger hearts; however, in the older hearts, the receptor was restricted to the subepicardial microvasculature, with a significantly lower density of TNF-R1 in the senescent subendocardium.

The functional significance of the changes in TNF-α receptor pathways in the aging cardiac microvasculature was then examined. On the basis of previous reports (12, 15) demonstrating that TNF-α promotes the expression of PDGF-B in endothelial cells in vitro, we hypothesized that TNF-α induction of PDGF-B would be impaired in the aging CMECs. In vitro stud-
ies confirmed that TNF-α induced the expression of PDGF-B in CMECs of the 4-mo-old rat heart but not in cells from the 24-mo-old hearts, Fig. 3A. In vivo TNF-α specifically induced the increase of PDGF-B in the younger hearts, while having minimal effect on the aging tissue (Fig. 3, B and C).

The functional impact of the senescent dysregulation of cardiac TNF-α receptor pathways contributed to the age-associated impairment in cardioprotective mechanisms. Indeed, the senescent alterations in the cardiac microvascular endothelial TNF-α receptor pathways contributed to the age-associated decrease in TNF-R1 in cardiac subendocardial microvasculature. Functional studies (10) demonstrated that this dysregulation in cardiac microvascular endothelial TNF-α receptor pathways contributes to the age-associated impairment in cardioprotective PDGF-B induction. Indeed, the senescent alterations in the cardiac actions of TNF-α resulted in increased mortality after coronary occlusion, which was reversed by the restoration of PDGF.

Previous studies (5, 8, 9, 11, 25, 35) have revealed that TNF-α mediates a diverse array of both beneficial and deleterious molecular and cellular cardiovascular responses. Potential protective actions of TNF-α include preconditioning of the ischemic heart (35), reducing hypoxic injury of cardiac myocytes (24), enhancing angiogenic activity (23, 42), and inducing PDGF pathways in endothelial cells (12, 15). The pathways governing these complex multicellular responses as well as TNF-α-mediated pathophysiology in the heart have not been fully elucidated but likely involve TNF-α secreted from cardiac myocytes (18, 39) regulating the function of endothelial cells expressing TNF receptors (44). The present studies demonstrate that short-term stimulation of intact TNF-α receptor pathways in the young cardiac microvasculature can induce expression

Fig. 4. TNF-α protects the young but not old rat heart from myocardial injury after coronary occlusion. A: representative cardiac histology after coronary occlusion: Masson’s trichrome staining (blue stain) for myocardial injury (14 days postcoronary occlusion) in 4- and 24-mo-old rat hearts treated with PBS control (4- and 24-mo-old, n = 4 and 10, respectively), TNF-α (4-mo-old, n = 4), PDGF-AB (4- and 24-mo-old, n = 8 and 10, respectively), and TNF-α/PDGF-AB (4- and 24-mo-old, n = 5, each). Analysis of the extent of myocardial injury in TNF-α-treated 24-mo-old rat hearts was not determined (ND) due to extensive mortality after coronary occlusion (Fig. 5). ND, not determined. B: quantification of the extent of injury in the different sets of rat hearts 14 days postcoronary occlusion. In the young heart, TNF-α, PDGF-AB, and the combination of TNF-α and PDGF-AB all reduced myocardial infarction size to a similar extent. Old rats pretreated with PDGF-AB and the combination of TNF-α and PDGF-AB were viable and had similar reductions in myocardial infarction size compared with control injections. *P < 0.05 infarction size in 4-mo-PBS vs. 4-mo-old TNF-α, 4-mo-old PDGF-AB, and 4-mo-old TNF-α/PDGF-AB, respectively. **P < 0.01 infarction size in 24-mo-PBS vs. 24-mo-old PDGF-AB and 24-mo-old TNF-α/ PDGF-AB, respectively.
myocytes (19, 24). Moreover, aging is associated with chronic increases in systemic levels of TNF-α (4, 28) and thus could compound vascular dysfunction due to apoptosis of older endothelial cells with altered TNF-R1 signaling pathways (17). Indeed, experimental models with sustained overexpression of TNF-α in the rodent heart result in marked left ventricular dysfunction and heart failure (5, 11), and the mortality after coronary occlusion observed in the TNF-α-treated older hearts may be due to the further enhancement of the senescent receptor pathways mediating this cardiovascular pathophysiology.

Therapies based on the cardioprotective role of TNF-α may have clinical utility in the younger heart, but novel strategies are required to optimize treatments of cardiovascular disease in older persons. Restoration of TNF-R1 expression in the aging cardiac microvasculature through gene therapy approaches has limited utility because the receptor itself can mediate viral-induced apoptotic pathways (16, 21, 43). Moreover, reexpression of the receptors may enhance the TNF-α proapoptotic pathways in the aging heart. Repopulation of the TNF-R1 endothelial cells with autologous or genetically matched endothelial precursor cells could overcome these limitations and potentially restore senescent vascular function. Alternatively, approaches aimed at decreasing or reversing the age-associated alterations in TNF-α and its receptor pathways may have significant applicability. To this end, previous studies (36) have demonstrated that estrogen protects endothelial cells from TNF-α-induced apoptosis and may be useful in preventing the senescent loss of TNF-R1 in cardiac endothelial cells. Similarly, identification of the critical genes downstream from TNF-α, potentially acting with PDGF-B induction, may allow for the development of cardiac-specific therapies that could have benefit for persons of all ages. Small molecules, including cyclic peptides, based on the structure and sequence of ΨY12, could provide novel strategies to selectively target cardioprotective pathways, potentially without the concomitant induction of apoptosis in older hearts. Moreover, such therapies could be combined with molecule approaches that are based on pathways that are upregulated in the senescent heart to develop therapies specifically tailored for the treatment and possible prevention of cardiovascular disease in older persons.

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DISCLOSURES

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REFERENCES


36. Spyridopoulos I, Brogi E, Kearney M, Sullivan AB, Ce-
trulo C, Isner JM, and Losordo DW. Vascular endothelial
growth factor inhibits endothelial cell apoptosis induced by tu-
mor necrosis factor-alpha: balance between growth and death
37. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA,
Holmes DR Jr, and Lerman A. Long-term follow-up of pa-
tients with mild coronary artery disease and endothelial dys-
38. Tahepold P, Valen G, Starkopf J, Ceslava K, Ailmer M, and
Vaage J. Pretreating rats with hyperoxia attenuates ischemia-
and Mann DL. Expression and functional significance of tumor
t necrosis factor receptors in human myocardium. Circulation 92:
40. Turner JR, Liu L, Fligiel SE, Jaszewski R, and Majumdar
AP. Aging alters gastric mucosal responses to epidermal growth
factor and transforming growth factor-α. Am J Physiol Gastro-
41. Weinsaft JW and Edelberg JM. Aging-associated changes in
vascular activity—a potential link to geriatric cardiovascular
42. Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki
H, and Kuwano M. Involvement of interleukin-8, vascular
endothelial growth factor, and basic fibroblast growth factor in
tumor necrosis factor alpha-dependent angiogenesis. Mol Cell
DT, Hsu HC, and Mountz JD. Hepatic DR5 induces apoptosis
and limits adenovirus gene therapy product expression in the
44. Zhang Y, Pasparakis M, Kollias G, and Simons M. Myo-
cyte-dependent regulation of endothelial cell syndecan-4 ex-
pression. Role of TNF-alpha. J Biol Chem 274: 14786–14790,
1999.