Exacerbation of myocardial injury in transgenic mice overexpressing FGF-2 is T cell dependent

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Departments of 1Physiology, 2Internal Medicine and Immunology, and 3Human Anatomy and Cell Science, University of Manitoba, Winnipeg R3E 3J7; and 4Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre, Winnipeg, Manitoba, Canada R2H 2A6

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Meij, Johanna T. A., Farah Sheikh, Sarah K. Jimenez, Peter W. Nickerson, Elissavet Kardami, and Peter A. Cattini. Exacerbation of myocardial injury in transgenic mice overexpressing FGF-2 is T cell dependent. Am J Physiol Heart Circ Physiol 282: H547–H555, 2002; 10.1152/ajpheart.01019.2000.—Fibroblast growth factor-2 (FGF-2) is cardioprotective when added exogenously, stimulates cardiac myocyte proliferation, and is a mediator of tissue repair after injury. Furthermore, transgenic (TG) mice overexpressing FGF-2 in cardiac muscle demonstrate increased resistance to injury in an isolated heart model of ischemia-reperfusion. We investigated how increasing the endogenous FGF-2 levels in the heart affects the extent of myocardial damage induced by isoproterenol in vivo. Histopathological evaluation of hearts after intraperitoneal injection of isoproterenol yielded significantly higher scores for myocardial damage in FGF-2 TG lines compared with non-TG mice. After 1 day, FGF-2 TG mouse hearts displayed more cellular infiltration correlating with increased tissue damage. Immunostaining of non-TG and FGF-2 TG mouse hearts showed the presence of leukocytes in the infiltrate, including T cells expressing FGF receptor-1. Treatment of mice with T cell suppressors cyclosporin A and anti-CD3ε significantly decreased the level of myocardial injury observed after isoproterenol and equalized the histopathology scores in FGF-2 TG and non-TG hearts. These data demonstrate a direct T cell involvement in the response to isoproterenol-induced injury in vivo. Moreover, the findings indicate that the exacerbation of myocardial damage in FGF-2 TG mice was dependent on T cell infiltration, implicating FGF-2 in the inflammatory response seen in cardiac tissue after injury in vivo.

myocardium; lymphocytes

FIBROBLAST GROWTH FACTOR-2 (FGF-2) is a polypeptide that can induce coronary angiogenesis and vasodilation (2) and is able to stimulate cardiac myocyte DNA synthesis in vitro (21, 45). FGF-2 is synthesized and released by several cell types in the heart, including cardiac myocytes, and exerts most of its effects in a paracrine and autocrine manner through binding to members of a receptor tyrosine kinase family, FGFR-1 (19–21, 45). Receptor as well as ligand are essential for embryonic development of heart and vasculature but may have different functions in the adult heart (2, 19–21, 45). Many findings, notably the enhanced release of FGF-2 on injury, point to an important role in healing mechanisms (10, 21, 35, 53). Healing after myocardial injury is a complex process involving the staged interactions between resident cells and infiltrating cells (granulocytes, macrophages, and lymphocytes) via a network of chemical signals (8, 9, 12, 53). With its angiogenic and mitogenic activities, FGF-2 is likely engaged in this network, but it is not clear how and at what stage(s) FGF-2-responsive cells are involved. FGF-2 plays an unfavorable role in the vascular response to injury by promoting neointimal hyperplasia and transplant atherosclerosis (7, 31). However, the reported protective effects of exogenously added FGF-2 on the myocardium (6, 13, 22, 25, 37, 38, 42, 49, 56), as well as through endogenous overexpression in isolated transgenic (TG) mouse hearts (44), suggest a beneficial role in the cardiac response to injury.

In the present study, we investigated whether an increase in the endogenous levels of FGF-2 in the heart can affect the extent of myocardial damage after injury in vivo. To this end, we used our previously characterized TG mice overexpressing the 18-kDa form of rat FGF-2 in the heart (44) and induced myocardial injury by the injection of isoproterenol (IsP) (41, 48, 57). A previous study (36) indicated a role for FGF-2 in IsP-induced myocardial injury. Our results showed a greater level of tissue damage corresponding to more cellular infiltration in two independent lines of TG mouse hearts overexpressing FGF-2. The damage was reduced by T cell suppression using cyclosporin A (CsA) or anti-CD3ε treatment. Thus our data implicate FGF-2 in the T cell-mediated inflammatory response seen in cardiac tissue after injury.

MATERIALS AND METHODS

Animals. Two homozygous TG mouse lines (nos. 5309 and 5323) were generated by pronuclear injection of CD-1 mouse eggs with a modified rat FGF-2 cDNA coding specifically for the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
18-kDa FGF-2 directed by the Rous sarcoma virus promoter (40, 44), which targets striated muscles in vivo (5, 16, 44). TG FGF-2 mRNA and protein expression were characterized as described elsewhere (3, 20, 21). The level of 18-kDa FGF-2 was increased 34- and 9-fold in FGF-2 TG lines 5323 and 5309, respectively, as assessed by densitometric scanning (44 and data not shown). Age-matched CD-1 mice were used as non-transgenic (non-TG) controls. A TG mouse line generated using the prostate-specific probasin gene promoter fused to the bacterial gene coding for chloramphenicol acetyl transferase (PB-CAT), a transgene unrelated to FGF-2, was used as an unrelated TG mouse control and was a generous gift from Dr. J. G. Dodd (University of Manitoba). All procedures were done in accordance with the guidelines of the Canadian Council on Animal Care.

**Induction and assessment of myocardial injury.** The experiments were carried out on several litters and both sexes of homozygous FGF-2 TG (lines 5309 and 5323) mice, and each experiment included age- and sex-matched normal (non-TG) CD-1 mice. Adult (6–10 wk old) mice were given a single intraperitoneal injection of l-isoproterenol HCl (IsP) (Sigma-Aldrich Canada; Oakville, Ontario, Canada) at a dose of 80 or 160 mg/kg body wt (41). Sham-treated mice were given the equal volume of 4 µL/g body wt vehicle (sterile saline). Mice were euthanized at 6 h and at 1 and 4 days after IsP injection. Cardiac ventricular tissues were fixed in 10% phosphate-buffered formalin (pH 7.4) and embedded in paraffin. Hearts were sectioned completely from apex to base. Grading and morphometric assessments were done on coded samples by an observer blind to treatment and mouse line.

For morphometric analysis, five H&E-stained sections (7 µm) corresponding to equivalent regions of each heart were assessed from four mice per treatment group. Lesions, associated with interstitial spaces containing infiltrating nuclei and/or myocyte disarray, were identified in five areas (each 0.3 mm²) per section and outlined using the “lasso” and “fill” features in Adobe Photoshop version 5.0. The number and volume of lesions per section (5 × 0.3 mm² × 7 µm) were determined using ImageTool version 2.0, and the results were expressed as the average number and percentage volume assessed per heart.

**Assessment of type and number of cells.** The cellular composition in areas of damage was determined in the LV tissues of FGF-2 TG (n = 6) and non-TG (n = 6) mice 1 day after isoproterenol injection. Heart weight-to-body weight ratios (means ± SE; n = 6–9) are expressed as a percentage of the mean value of the matching saline-treated group. *P < 0.01 vs. sham-treated FGF-2 TG group.

**Fig. 1.** Effects of isoproterenol (IsP)-induced myocardial injury on the heart weight-to-body weight ratios of nontransgenic (non-TG) and fibroblast growth factor-2 (FGF-2) TG mice. Mice were injected with saline (dashed lines), 80 mg/kg body wt IsP (open symbols), or 160 mg/kg body wt IsP (closed symbols) and euthanized 1, 7, or 28 days (d) later (see MATERIALS AND METHODS). Heart weight-to-body weight ratios (means ± SE; n = 6–9) are expressed as a percentage of the mean value of the matching saline-treated group. *P < 0.01 vs. sham-treated FGF-2 TG group.

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Values are means ± SE; n, scores of the indicated no. of surviving mice. Mice were injected with the indicated dose of isoproterenol (IsP) and euthanized at 1 day, and the left ventricular (LV) pathology scores were determined as described in MATERIALS AND METHODS. None refers to the LV pathology scores in CD-1 mice expressing prostate-specific probasin gene promoter fused to the bacterial gene coding for chloramphenicol acetyl transferase (PB-CAT), a transgene unrelated to fibroblast growth factor-2 (FGF-2) (43), which were 1.59 ± 0.16 (n = 3) 80 mg/kg body wt IsP and 2.45 ± 0.33 (n = 3) after 160 mg/kg body wt IsP compared with nontransgenic (non-TG) values. RSVp.metFGF, modified rat FGF-2 coding specifically for 18-kDa FGF-2 directed by the Rous sarcoma virus promoter (32, 36). *P < 0.01 vs. sham-treated group of the same line; †P < 0.05 and ‡P < 0.001 vs. non-TG group (80 mg/kg body wt IsP); §P < 0.05 and ¶P < 0.01 vs. non-TG group (160 mg/kg body wt IsP).

**Table 1. Left ventricular pathology scores 1 day after isoproterenol injection**
treatment with IsP (160 mg/kg). Comparable regions in each heart were assessed microscopically. Specifically, in an equatorial section, six fields in the subendocardial layer, roughly at the positions of the “even hours” on a dial, were analyzed. On the basis of morphology and H&E staining, cells could be distinguished as cardiac myocytes, neutrophils (2–5 nuclear lobes, ~9–15 μm diameter), macrophages (nucleus is oval or kidney shaped; ~15–80 μm diameter; tinting of cytoplasm due to phagocytosis of dying cells and cellular debris, including erythrocytes), and lymphocytes (~8–16 μm diameter; round or oval nucleus with a thin rim of cytoplasm) (9, 18).

Immunosuppression. Twelve FGF-2 TG (line 5323) and twelve non-TG mice of both sexes were divided into three groups and treated daily for 4 days before the injection of 160 mg/kg IsP with 1) an oral dose of 75 mg/kg CsA (Neoral; Novartis Pharmaceuticals; East Hanover, NJ) (1); 2) an intraperitoneal injection of 50 μg hamster monoclonal antibodies to the mouse CD3 (clone 145-2C11, ATCC CRL_1975) (15); or 3) vehicle. Treatments were continued for another 4 days after IsP administration, after which the animals were euthanized. The doses of CsA and CD3 antibodies were based on those shown to be immunosuppressive in mice in vivo (1, 15). Hearts were paraffin-embedded and both LV pathology scores and morphometric assessment of lesion size were determined as described in Induction and assessment of myocardial injury.

Fig. 2. Analysis of cardiac myocyte array and myocardial lesion(s) in non-TG (A and B) and FGF-2 TG (C–E) mice, 1 day after IsP injection. Mice were injected with either 160 mg/kg body wt IsP (A–D) or saline (E) and euthanized 1 day later. Paraffin-embedded ventricular sections were stained for nuclei and cytoplasm with hematoxylin and eosin (H&E), respectively. Ventricular sections from age-matched FGF-2 TG mice treated with saline and stained for H&E are shown for comparison. Bar = 50 μM.

Fig. 3. Effects of IsP-induced myocardial injury on lesion size and numbers in non-TG and FGF-2 TG mouse hearts 1 day after IsP injection. Mice were injected with 160 mg/kg body wt IsP and euthanized 1 day postinjection. Paraffin-embedded ventricular sections from non-TG and FGF-2 TG mice were stained with H&E and morphometrically assessed for lesion numbers and size (see MATERIALS AND METHODS). Five areas per ventricular section and five sections per heart were assessed per animal. Lesion volume (mean) is expressed as a percentage of the total cardiac ventricular volume assessed (5.25 × 10^7 μm^3). Bars are means ± SE. *P < 0.05 vs. non-TG mice.

Fig. 4. Correlation between density of infiltrating cells and level of tissue damage in non-TG (top bar) and FGF-2 TG (bottom bar) mice 1 day after IsP injection. Sections from mouse heart equatorial areas were analyzed (see MATERIALS AND METHODS). Stacked bars represent total number per field (means ± SE) of nonmyocytes (right) and cardiac myocytes (left). *P < 0.05 vs. non-TG mice.
Immunofluorescence microscopy. To detect FGFR-1 and/or T lymphocytes, heart and spleen cryosections were acetone fixed for 10 min, hematoxylin stained for 5 s, blocked with 2% normal goat serum-1% bovine serum albumin-phosphate-buffered saline (BSA-PBS) for 30 min at room temperature, and incubated either 1) overnight at 4°C in 1% BSA-PBS containing rabbit anti-Flg (C-15) (1:200) and rat anti-mouse CD4 (L3T4) (1:50) (18), or 2) 2 h at room temperature in 1% BSA-PBS containing rabbit anti-human CD3 (1:25; DAKO Diagnostics; Mississauga, Ontario, Canada), followed by Cy3 donkey anti-rabbit IgG (1:50; Jackson Immunoresearch Laboratories; West Grove, PA) to reveal FGFR-1 or CD3. After the sections were extensively rinsed to remove free anti-rabbit IgG, biotinylated rabbit anti-rat IgG (1:500; Vector Laboratories) and fluorescein isothiocyanate-streptavidin (American Life Sciences; Oakville, Ontario, Canada; 1:20) were applied to reveal CD4. Control sections without anti-Flg (C-15) were included to rule out a cross reaction of the Cy3 anti-rabbit IgG and the rabbit anti-rat IgG (Vector). Each experiment also included control sections that were treated equally except that buffer replaced all primary antibodies. For CD4/FGFR-1 colocalization studies, counterstaining of nuclei was done by dipping slides in 125 nM bisbenzimide (Hoechst no. 33258, Sigma) for 10 min. The stained sections were mounted in fluorescent mounting medium (DAKO Diagnostics Canada) or Crystalmount mounting media and examined by epifluorescence or confocal microscopy.

Statistical analysis. Data are expressed as means ± SE. Statistical significance was determined by Student’s t-test or nonparametric Mann-Whitney test. For multiple comparisons, we used analysis of variance, followed by either the Bonferroni (Table 1) or the Tukey-Kramer test (Instat, GraphPad Software; San Diego, CA). Values of P < 0.05 were considered significant.

RESULTS

Myocardial injury. In non-TG mice, a positive correlation between IsP dose (0–320 mg/kg body wt ip) and the level of LV injury was observed (Table 1). Doses of 80 and 160 mg/kg IsP that still yielded 100% survival at day 1 were used to compare effects in TG mice overexpressing FGF-2 and matching non-TG mice. There was no obvious difference between FGF-2 TG and non-TG mice regarding the acute effects of IsP administration. It was noted that at each dose, one of the FGF-2 TG mice died at ~25 days after IsP injection. To assess the response to IsP injection, the heart weight-to-body weight ratios of each IsP-injected group were expressed as a percentage of the value in the matching sham-treated group. A significant 54% increase was noted in the FGF-2 TG mice at 28 days after injection of 160 mg/kg IsP (Fig. 1). The effects were similar in the FGF-2 TG lines 5309 and 5323, indicating that the hypertrophic response after IsP was stronger in both FGF-2 TG mouse lines compared with non-TG mice.

Histological examination of mouse hearts 1 day after IsP injection showed differences in the extent of myocardial injury in FGF-2 TG and in non-TG mice (Fig. 2). Extensive myocyte disarray was observed in IsP- versus saline-treated FGF-2 TG mouse hearts (Fig. 2, A and B) and the larger contiguous lesions in the IsP-treated non-TG hearts (Fig. 2A). There was also a striking contrast between the small focal lesions in the IsP-treated non-TG hearts (Fig. 2B) and the larger contiguous lesions in the IsP-treated FGF-2 TG hearts (Fig. 2D). The larger IsP-induced lesions in the FGF-2 TG mice were reflected in significantly higher LV pathology scores (Table 1) as well as morphometric assessment, which indicated significance about six- and twofold increases in lesion size and number 1 day after 160 mg/kg IsP treatment (Fig. 3).

Identification of T cells in myocardial lesions. At day 1, cellular infiltration was apparent in all areas of cardiac myocyte damage (Fig. 2). As shown in Fig. 4, there were 54% more damaged cardiac myocytes and a comparable 40% more infiltrating cells in the same areas of FGF-2 TG versus non-TG mouse hearts. Neutrophils, macrophages, and lymphocytes could be recognized among the infiltrating cells (Fig. 5). Monocytes/macrophages and activated macrophages were also detected in myocardial lesions by immunostaining with MOMA-2 (1:100; Serotec; Kidlington, Oxford, UK) and rabbit antibodies to allograft inflammatory factor-1 (1:500; gift from Dr. Mary Russell, Harvard School of Public Health, Boston, MA), respectively (data not shown). The presence of T lymphocytes was indicated by arrows.

Fig. 5. Assessment of infiltrating cells in myocardial lesions 1 day after IsP injection. Paraffin-embedded cardiac ventricular sections were stained with H&E. Identification of neutrophils (A), macrophages (B), and lymphocytes (C) in H&E sections from FGF-2 TG mice was done (see MATERIALS AND METHODS), and examples of each are indicated by arrows. Bar = 10 μM.
demonstrated by immunofluorescence detection of CD3+ and/or CD4+ cells in IsP-injected FGF-2 TG mouse hearts at 1 day and 4 days posttreatment (Figs. 6 and 7). FGFR-1 positive cells in the lymphocyte population of FGF-2 TG as well as non-TG mouse hearts was indicated by costaining with FGFR-1 and CD4 antibodies (Fig. 7).

Effect of immunosuppression on IsP-induced myocardial injury. To determine the relative role of T cells in the extent of myocardial damage, mice were treated for 4 days before and 4 days after IsP injection with either anti-CD3ε or CsA to prevent T cell activation. Effects at 4 days post-IsP treatment were evaluated by LV pathology score and morphometric assessment of lesion size. Assessment of LV pathology scores in FGF-2 TG mice (line 5323) indicated that IsP-induced myocardial injury was decreased from a value of 4.4 ± 0.4 to 1.2 ± 0.6 and 1.4 ± 0.6 with CsA and anti-CD3ε treatments, respectively (n = 3), which was comparable to non-TG values (0.9 ± 0.1 with CsA and 1.3 ± 0.3 with anti-CD3ε, n = 3). A significant reduction in damage through immunosuppression, down to non-TG

Fig. 6. Detection of CD3-positive cells in myocardial lesion areas by confocal microscopy. FGF-2 TG mice were injected with 160 mg/kg IsP and euthanized 1 or 4 days later. Hearts were subsequently frozen in optimum cutting temperature (OCT) compound, cryosectioned (7 μM), and acetone fixed. Ventricular sections from 1-day (A–C) and 4-day (D–F) animals were stained with hematoxylin to identify nuclei (A and D) and CD3 antibodies to identify T cells (B and E). Colocalization of CD3-positive cells among infiltrating nuclei in myocardial lesions are indicated by arrows (C and F). A spleen section stained for CD3 (G) and ventricular section from a FGF-2 TG mouse without primary antibody incubation (H) are shown as positive and negative controls, respectively. Bar = 20 μM (A–F) and 80 μM (G and H).
levels, was also observed in FGF-2 TG mouse hearts by morphometric assessment of lesion size (Fig. 8).

DISCUSSION

The current study shows that overexpression of FGF-2 in the TG mouse heart leads to a greater extent of IsP-induced myocardial injury via an infiltrating T cell-dependent mechanism. The greater damage to FGF-2 TG compared with matching non-TG mice was evidenced by cardiac myocyte disarray as well as increased lesion size and number (Table 1 and Figs. 2–4). In addition, the significant level of hypertrophy (Fig. 1) may be viewed as compensatory for the greater extent of damage in the FGF-2 TG mice. We cannot exclude, however, an added hypertrophic effect on the myocytes due to higher FGF-2 levels. FGF-2 has been implicated in cardiac hypertrophy in vitro (39) and in vivo (43). The presence of lymphocytes (Figs. 5 and 6) as well as the reduction in injury to the same level in both FGF-2 TG and non-TG mice treated with CsA or anti-CD3 antibodies indicates that the damage was linked to infiltration and was not a direct effect (acute or chronic) on the cardiac myocytes (Fig. 8). Moreover, increased cellular infiltration in TG mouse hearts (Figs. 2 and 4), as well as the elimination of the greater extent of myocardial damage in TG mice by T cell suppression (Fig. 8), suggests an effect of FGF-2 on T lymphocytes. This is supported further by the detection of FGFR-1 in T cells present in the infiltrate (Fig. 7).

The increased myocardial damage seen in FGF-2 TG mice seemed in conflict with results of other studies, in which FGF-2 was added in vivo. In dogs, pigs, and humans, FGF-2 treatment improved heart function, reduced infarct size, and increased blood flow in areas of ischemia (13, 25, 49, 56). Better recovery from ischemia-reperfusion damage in isolated FGF-2-perfused rat and mouse hearts was also observed (37, 38, 44). In all of these studies, heart cells were only transiently exposed to the exogenously added FGF-2. Recently, we (44) were also able to detect significant protection from ischemia-reperfusion injury in isolated FGF-2 TG versus non-TG mouse hearts, reflected by increased cardiac myocyte viability. As in the current study, the endogenous overproduction ensures that TG mouse hearts are exposed to extra FGF-2 during development (5). However, this does not appear to be through an increase in systemic FGF-2 levels in these mice (44). Rather, FGF-2 is released locally in the heart on a beat-to-beat basis and surges on injury (4, 10, 23, 44). The presence of more FGF-2 is expected to be cardioprotective. The results of our current study suggest,
however, that any direct protective effect on the myocardium may be offset by an augmented inflammatory response (Figs. 5–8).

An acute inflammatory response involving leukocytes is recognized as a major causative factor in myocardial injury (8, 12, 54). Characterization of the infiltrating cells at day 1 confirmed the presence of leukocytes including T lymphocytes (Figs. 4–7). These findings support the idea that the extent of injury corresponded to the extent of cardiac myocyte damage and infiltration at day 1 (52). Suppression of T cells with CsA or anti-CD3ε antibodies greatly reduced the level of myocardial injury seen after IsP treatment (Fig. 8). CsA inhibits calcineurin, the phosphatase that activates the transcription factor, nuclear factor of activated T cells (NFAT) (14). In T cells, NFAT inhibition suppresses lymphokine production and T cell activation, but NFAT also affects cardiac myocyte gene transcription (32). In a TG rat model of angiotensin II-induced end-organ damage, CsA was shown to reduce injury through inhibition of inflammatory pathways (29). In the case of antibodies to CD3ε, a protein existing only as a component of the T cell receptor complex (18), immunosuppression results from their ability to cross-link the T cell receptor and thereby inhibit maturing precursor T cells specifically (15). In conclusion, the observation that the extent of injury was reduced to the same level in both FGF-2 TG and non-TG mouse hearts after immunosuppression with either CsA or anti-CD3ε antibodies indicates that a component of IsP-induced myocardial injury is T cell dependent (Fig. 8). If the cardiac injury was T cell independent, and related to a direct effect of the FGF-2 on the myocardium or cardiac myocytes themselves, for example, then the removal of T cells would be expected to maintain the differences in damage after IsP treatment. This was not the case (Fig. 7). Finally, it is unlikely that exposure to additional “endogenous” FGF-2 is damaging to the myocytes in view of the increased cardiac myocyte viability observed in isolated FGF-2 TG mouse hearts (44).

We detected CD4+FGFR-1+ cells in FGF-2 TG as well as non-TG mouse hearts (Fig. 7), suggesting a link between FGF-2 signaling and T cells. This raises the possibility that FGF-2 release at the time of injury contributes to the “normal” inflammatory damage caused by T cell infiltration. The FGF-2 is presumably released from the extracellular matrix as well as damaged and/or contracting myocytes. This would be consistent with an increase in the availability of FGF-2 postinjury in the TG mice and a corresponding increase in the level of infiltrating T cells and damage to the myocardium. There are at least two ways in which FGF-2 might affect T cell infiltration in non-TG mice as well as provoke greater infiltration and damage in the FGF-2 TG mice. This includes an effect on the recruitment and/or on the expansion of these lymphocytes. With regard to recruitment, FGF-2 is a known chemotactrant affecting cell attachment and migration (2, 35, 53) and accumulates at sites of myocardial injury induced by IsP (36). The expected higher concentration gradient of FGF-2 in the FGF-2 TG mice could attract a greater influx of T cells. Regarding expansion, it is likely that inflammatory leukocytes responded to FGF-2 in a proliferative manner because many infiltrating cells, including CD4+ T lymphocytes, were found to express FGF receptor-1 (Fig. 7). The costimulatory effects of FGF-1 and -2 on expansion of FGF receptor-1-expressing T cells have been demonstrated in vitro (31, 54). As well, FGF receptor-1-bearing T cells were implicated in the pathogenesis of rheumatoid arthritis and heart transplant atherosclerosis in humans (7, 31).

Although we did not assess the mechanism of cardiac myocyte death, loss can occur in hypoxia and reperfusion injury by apoptosis as well as necrosis (11, 24, 26, 27, 46, 47). Reperfusion is associated with acute increases in free radical production and increases in intracellular calcium, both potent inducers of apoptosis (11, 17). Thus an increase in apoptosis might possibly contribute to the level of cardiac injury observed in IsP-treated FGF-2-TG mice. Although FGF-2 has been linked with apoptosis (30), the majority of studies appear to support an anti-apoptotic effect. Specifically, protein kinase C-dependent regulation of intracellular free calcium levels (28) and effects on nitric oxide-induced apoptosis has been implicated in the anti-apoptotic effect in noncardiac systems (33, 51). Involvement of an FGF-2 interacting-factor, which has anti-apoptotic properties, has also been reported (50).

Thus while it is clear that FGF-2 can be cardioprotective, our data raise the possibility that in the event of myocardial injury, FGF-2 released or available to damaged sites stimulates the inflammatory response, a function that was amplified in the presence of excess FGF-2 in the TG mouse. A further role for FGF-2 in remodeling or any compensatory hypertrophy for the damaged myocardium may complete the response. Regardless, our study demonstrates that FGF-2 overexpression in the heart can exacerbate T cell-mediated injury in vivo.

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