A potential mechanism of vasodilation after warm heart surgery

The temperature-dependent release of cytokines

Peripheral vasodilation is a common feature of warm heart surgery and creates clinical concerns when pressor agents become necessary because of the potential for some of these drugs to adversely affect flow through newly engrafted arterial and venous bypass conduits. The possible role of a temperature-dependent production of cytokines in the pathogenesis of this vasodilation was investigated in a two-part study. In part I, lipopolysaccharide-activated peritoneal rabbit macrophages ($5 \times 10^6/\text{ml}$) were incubated at 30° or 37° C up to 9 hours and the concentration of tumor necrosis factor released in the supernatant was serially measured by a bioassay. Tumor necrosis factor production was found to increase over time for each of the two temperatures of incubation but was significantly higher throughout the observation period in normothermic experiments than in those done at 30° C. Part II was a prospective clinical study involving 30 patients who underwent either cold (core temperature 28° to 30° C, $n = 15$) or warm (37° C, $n = 15$) cardiopulmonary bypass and in whom serum levels of tumor necrosis factor α , interleukin-1 β , and interleukin-6 were measured by enzyme-linked immunosorbent assays at 2, 4, 10, and 24 hours after bypass. Cytokine levels were found to be consistently higher in patients having normothermic bypass. Differences between the two groups were significant 2 hours after bypass for tumor necrosis factor α and interleukin-6 ($p < 0.02$ and $p = 0.0001$, respectively) and 4 and 10 hours after bypass for interleukin-1 β ($p < 0.01$ and $p < 0.04$, respectively). The incidence of vasodilation necessitating vasopressor support was twofold higher in the normothermic group (six patients versus three in the hypothermic group). Taken as a whole, patients supported by pressor agents had significantly higher cytokine levels after bypass than those who did not require pressor therapy. Our results suggest that vasodilation occurring with warm heart operation is, at least partly, mediated by a temperature-dependent release of cytokines. Vasodilation might therefore be mitigated by simply allowing the core temperature to drift during bypass. Our recent clinical experience suggests that this "tepid" heart surgery (32° to 34° C) effectively blunts most of the vasodilatory response to strictly normothermic bypass without compromising maintenance of myocardial aerobiosis during arrest. (J THORAC CARDIOVASC SURG 1994;107:293-9)

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Received for publication Feb. 25, 1993.

Accepted for publication June 16, 1993.

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0022-5223/94 \$1.00 + .10 12/1/50731

The recently introduced concept of warm heart surgery,¹ which involves both normothermic cardiopulmonary bypass (CPB) and continuous heart perfusion with normothermic cardioplegic blood, has generated several clinical studies that have primarily focused on the myocardial metabolic consequences of aerobic arrest,² the particular benefits of this type of protection in high-risk patients,³⁻⁵ and the optimal technical modalities of cardioplegia delivery.^{6,7} Comparatively, fewer studies have addressed the issues raised by maintenance of normo-

thermia during bypass.^{8,9} One of these issues relates to the frequent occurrence of intraoperative and postoperative peripheral vasodilation, which creates clinical concerns when vasopressors become necessary, because some of these drugs can compromise flow through newly constructed arterial and venous bypass grafts.¹⁰

It has been recognized for several years that CPB elicits an inflammatory response in the genesis of which activation of the complement system plays a pivotal role.^{11,12} However, other inflammatory mediators are known to be liberated during bypass in man^{13,14} and, in this setting, a growing interest is currently paid to cytokines in view of the documented involvement of these peptides in both inflammatory and immune responses.^{13,15-17} One of the main targets of cytokines, and more specifically of tumor necrosis factor (TNF) and interleukin-1 (IL-1), is the vessel wall,¹⁸ where these mediators cause relaxation through the induction of nitric oxide synthase in smooth muscle cells.^{19,20} This observation makes cytokines attractive candidates for explaining, at least partly, peripheral vasodilation that occurs with warm heart operation. Testing of this hypothesis forms the subject of the present, two-part study. In part I, *in vitro* experiments were used to assess the effect of temperature on TNF production by endotoxin-activated rabbit peritoneal macrophages. Part II was a prospective clinical study designed to assess cytokine production and its effects on the prevalence of vasodilation in 30 patients undergoing either hypothermic or normothermic CPB for various cardiac operations.

Materials and methods

Experimental study. Male New Zealand white rabbits (2 to 2.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg intravenously). Elicited peritoneal macrophages were obtained by intraperitoneal injection of 40 ml sterile mineral oil and aseptic lavage of the peritoneum with 2% fetal bovine serum in RPMI 1640 medium 3 days later. Cells were then harvested and centrifuged twice for 10 minutes at 1000 *g* at 4° C and erythrocytes were lysed under hypotonic conditions. The remaining cells were resuspended in serum-free RPMI medium at a density of 5×10^6 cells/ml, plated (500 μ l) in 24-well microplates and incubated for 90 minutes at 37° C in 5% CO₂, as previously described.²¹ Nonadherent cells were removed and wells were replenished with 500 μ l of serum-free RPMI medium. The resulting cell population included more than 90% macrophages when examined by phase-contrast microscopy for cytomorphologic characteristics and Wright staining. More than 95% of these macrophages were viable, as assessed by trypan blue exclusion.

Macrophages (2.5×10^6 cells per well) were incubated for 1, 2, 3, 4, 6, or 9 hours at 30° or 37° C in the presence of endotoxin (lipopolysaccharide, 1 μ g/ml). The supernatants were collected at these various study points and stored at -80° C until measurement of TNF activity.

TNF activity was measured by an *in vitro* cell cytotoxicity assay with actinomycin D-treated murine fibroblast L-M cells (American Type Culture Collection, Rockville, Md.). L-M cells were plated into 96-well microtiter plates at 5×10^4 cells per well and incubated 24 hours at 37° C in 5% CO₂. After incubation, the medium was aspirated and 100 μ l of fresh medium was added to all wells. Recombinant human TNF α (specific activity 3×10^6 U/ng) was diluted into medium for standards. Fifty microliters of standards or samples plus an additional 50 μ l of medium were pipetted in duplicate into the first column of wells and then serially diluted across the plate. One hundred microliters of medium containing 10 μ g/ml actinomycin D was added to all wells and the cells were incubated for 24 hours. Cytotoxicity was detected by a tetrazolium dye technique. The plates were read at 570 nmol/L on a microtiter plate reader (model 650, Dynatech Laboratories, Alexandria, Va.) against *n*-propyl alcohol blanks. A standard curve relating cell cytotoxicity to doses of recombinant human TNF α was used to quantify TNF activity in the supernatant.

Clinical study. Thirty consecutive patients undergoing extracorporeal circulation for valve or coronary artery bypass operations were prospectively entered into this study, which was approved by our institutional human experimentation committee. The patients were assigned to receive either cold or warm CPB according to their respective surgeons' practice. There was no significant difference between the two groups, in particular with respect to the age of the patients (53 ± 5 years in the cold group and 59 ± 5 years in the warm group [mean plus or minus standard error of the mean]) and to the CPB times (107 ± 8 and 131 ± 9 minutes, respectively).

Anesthesia was uniform in all cases and consisted of a standard combination of fentanyl citrate, flunitrazepam, and pancuronium bromide. CPB equipment consisted of a roller pump, a membrane oxygenator, and an arterial filter. Crystalloid solutions were used to prime the extracorporeal circuit unless patients had preoperative anemia (hemoglobin level less than 10.0 gm/dl), in which case packed red blood cells were added to the priming mixture (two patients in each group). Body temperature was monitored continuously with a nasopharyngeal probe. In patients assigned to receive cold CPB, the perfusate temperature was cooled to 28° to 30° C, whereas those in the warm group were kept between 35° and 37° C. Pump flows were maintained at 2.2 L/min/m² except during cold bypass in which they were reduced by an average of 1 L below normothermic levels. Sodium nitroprusside, isoflurane (1%), and phenylephrine hydrochloride were used as required to maintain systemic perfusion pressures between 50 and 70 mm Hg. Administration of heparin before cannulation and subsequent neutralization after bypass with protamine sulfate were accomplished in a standard fashion.

In the cold group, myocardial protection relied on a single dose of 1 L of a cold (4° C) crystalloid cardioplegic solution that was administered through the aortic root after the application of the aortic crossclamp. Additional topical hypothermia was obtained by wrapping the heart in sponges soaked in cold saline. In the warm group, myocardial protection was provided by continuous perfusion with normothermic cardioplegic blood. Arrest was initially induced through the aortic root and subsequently maintained by continuous retrograde coronary sinus perfusion according to a previously described technique.⁷ The cardioplegic formulation was limited to potassium and magnesium and was concentrated in a small volume (16 mEq KCl and

3 mEq MgCl₂ in a 20 ml ampule). The cardioplegic content of these ampules was continuously added to a separate blood circuit originating from a sideport of the oxygenator and connected distally to the cardioplegia infusion catheter. Delivery of the cardioplegic ampules was made by means of an electrically driven syringe whose infusion rate was adjusted on-line so as to keep the heart continuously arrested.

Whole blood samples (10 ml) were drawn from the radial artery catheter after induction of anesthesia but before sternotomy and subsequently at intervals of 2, 4, 10, and 24 hours after the institution of CPB. Blood was immediately centrifuged and plasma was stored at -20°C until assayed. The levels of TNF α , IL-1 β , and interleukin-6 (IL-6) were determined in duplicate by enzyme-linked immunosorbent assays with commercially available kits (British Biotechnology, Oxford, United Kingdom). The interassay and intraassay variations for TNF α , IL-1 β , and IL-6 were less than 8% and less than 10%, less than 9% and less than 9%, and less than 8% and less than 5%, respectively. All postbypass data are expressed corrected for hemodilution occurring during CPB.

Intraoperative and postoperative records of all patients were carefully reviewed for the occurrence of cardiac-related and noncardiac-related adverse events. Peripheral vasodilation was defined as the need for purely vasoconstrictive drugs (such as phenylephrine or norepinephrine) because of low systemic pressures. During bypass, vasodilation was considered to have occurred when mean arterial pressure fell below 50 mm Hg despite high pump flow rates. After bypass, the diagnosis of vasodilation was based on a fall in systolic arterial pressure below 80 mm Hg coexistent with a correspondingly low diastolic pressure (differential systemic pressure 40 mm Hg or greater) and a decreased systemic vascular resistance index (less than 1000 dynes/sec/cm⁻⁵/m²), as documented by standard hemodynamic measurements. All instances of vasopressor therapy, including infusion drips of short duration, were tabulated.

Statistical analysis. A two-tailed unpaired *t* test was done for comparison between the experimental groups. On the other hand, a nonparametric Kendall's rank correlation test was used for comparison between the two clinical treatment groups. Significance was set at the 0.05 level. Results are expressed as the mean plus or minus the standard error of the mean.

Results

Experimental study. As shown in Fig. 1, endotoxin-activated macrophages released TNF in both a time-dependent and temperature-dependent fashion. Production of TNF was significantly greater at 37°C than at 30°C at each of the study points. However, the patterns of TNF release were different between the two groups. In the hypothermic group, TNF levels remained very low up to 3 hours of incubation and rose steadily thereafter. Conversely, in normothermic experiments, TNF α levels increased steeply between 1 and 6 hours of incubation and tended to level off thereafter.

Clinical study. TNF α and IL-1 β were not detectable in either group before bypass. Only trace levels of IL-6 were detectable at baseline and these were not significantly different between the two groups.

Fig. 1. Time course of TNF production by rabbit peritoneal macrophages incubated in presence of lipopolysaccharide (1 $\mu\text{g}/\text{ml}$) in RPMI 1640 medium at 30°C or 37°C . Supernatants were assayed on L-M cells to determine TNF activity. Each group consisted of eight experiments. Results are expressed as mean plus or minus standard error of mean.

Cytokine levels after bypass were consistently higher in the normothermic group (Fig. 2). In both groups, TNF α levels peaked 2 hours after the institution of bypass ($p < 0.02$ between cold and warm groups) and declined thereafter. However, at the 4-hour postbypass study point, patients receiving normothermic CPB still produced measurable amounts of TNF α whereas TNF α values had already returned to almost undetectable levels in the hypothermic group. Levels of IL-1 β yielded similar kinetic patterns except that peak values were recorded later (4 hours) in the interval after bypass, in particular in the normothermic group. Differences between the two groups for IL-1 β values were significant at both 4 and 10 hours after the onset of bypass ($p < 0.01$ and $p < 0.04$, respectively). The IL-6 response to CPB was both quantitatively more pronounced and more prolonged over time. IL-6 levels peaked 4 hours after the initiation of bypass and subsequently declined in patients receiving hypothermic CPB, whereas they tended to remain elevated throughout the observation period in those who had been kept normothermic during bypass. Although mean values of IL-6 were markedly higher in the normothermic group than in the hypothermic group, statistical analysis failed to show significant between-group differences other than at 2 hours after initiation of bypass ($p = 0.0001$).

The incidence of intraoperative and/or postoperative pressor therapy was twofold higher in the normothermic group inasmuch as six patients in this group and three patients receiving hypothermic CPB required vasoactive drugs. When all patients of the two groups ($n = 30$) were

Fig. 2. Kinetics of release of TNF α , IL-1 β , and IL-6 in patients having undergone normothermic (35° to 37° C) or hypothermic (28° to 30° C) CPB. Each group consisted of 15 patients. Results are expressed as mean plus or minus standard error of mean.

stratified on the basis of the need for pressor therapy, patients receiving support ($n = 9$) were found to have significantly higher cytokine levels than those not receiving support ($n = 21$). As shown in Fig. 3, differences in TNF α and IL-6 levels between these two subsets of patients were significant at 2 and 4 hours after bypass ($p < 0.01$ and $p < 0.03$, and $p < 0.03$ and $p < 0.01$, respectively). Differences in IL-1 β levels between pressor-supported patients and their untreated counterparts were significant at 4 hours after bypass ($p < 0.01$).

Discussion

Clinical relevance of the study. Peripheral vasodilation is a common observation during and after normothermic CPB. Thus Christakis and associates⁹ have recently reported a threefold higher incidence of intraoperative vasopressor support in patients undergoing warm heart operation compared with that in those who had received conventional hypothermic CPB. There is no question that many factors, such as hemodilution,²² anesthetic drugs, or preoperative medical therapies, can contribute to the fall in systemic vascular resistance occurring with warm heart operation. However, assuming that (1) cytokines like

TNF, IL-1, and IL-6 are potent vasodilators,¹⁸ and that (2) secretion of these cytokines by activated monocytes and macrophages can be induced by endotoxin and complement-derived anaphylatoxins,^{15,18} which are known to be released during CPB in man,^{11,17,23,24} we tested the hypothesis that peripheral vasodilation commonly seen in normothermically perfused patients could be mediated by a temperature-dependent cytokine production. Such an investigation was thought to be clinically relevant in that a better understanding of the mechanisms of bypass-induced vasodilation should help in developing therapeutic strategies designed to eliminate this negative feature of warm heart surgery.

Interpretation of results. Our experimental results clearly show that TNF production by endotoxin-activated macrophages is indeed temperature-dependent and increases over time. It is noteworthy that differences in TNF production by endotoxin-activated macrophages incubated at 30° and 37° C were most significant during periods of incubation that are relevant to the time frames commonly achieved during clinical CPB, that is, between 2 and 4 hours. After 9 hours of incubation, the difference between the two experimental groups tended to become

Fig. 3. Influence of levels of TNF α , IL-1 β , and IL-6 on requirements for vasopressor therapy. Pressor-supported group consisted of 9 patients (6 having undergone normothermic bypass and 3 having undergone hypothermic bypass), and group not having pressor support consisted of remaining 21 patients. Results are expressed as mean plus or minus standard error of mean.

narrower (although still statistically significant), probably because activated macrophages incubated under hypothermic conditions were still able to secrete TNF at a time when the production activity of those exposed to normothermia was almost completely exhausted. These results are consistent with those of Haeffner-Cavaillon and coworkers¹⁵ who have previously demonstrated that IL-1 production by activated monocytes drastically decreased between 37° and 26° C. Additional evidence for the thermodependency of cytokine production can be found in the study of Lederman, Brill, and Murphy,²⁵ who have shown that the rate of IL-2 release by a T lymphoma cell line increases linearly with temperature over the range from 33° to 41° C. Not unexpectedly, a parallel regulatory effect of temperature has been observed at the level of interleukin-2 gene expression.²⁶ Taken together, these data support our observations that hypothermia can both delay the onset of cytokine secretion and reduce the magnitude of their production.

These experimental results are corroborated by our clinical findings because cytokine levels were significantly higher in patients kept normothermic during bypass than in those of the hypothermic group. Although the study was not randomized, the two groups were correctly

matched for the major preoperative and intraoperative variables so that these differences in cytokine production are likely to reflect the predominant influence of core temperature during the bypass period. Regardless of this temperature, TNF α levels peaked 2 hours after the onset of bypass, which is in accordance with the time interval required for activated macrophages and monocytes to produce TNF.^{17,18} The more delayed rise in IL-1 β and IL-6 is, in turn, consistent with the role of TNF as an initiator of the cytokine cascade.¹⁸ The impact of temperature on production of this cytokine was clearly reflected by our finding that peak TNF α levels were 10-fold higher in normothermic patients than in those who were cooled during CPB. In the latter group, TNF α values measured 2 hours after bypass were similar to those reported by Jansen and associates^{13,17} in patients undergoing a similar degree of cooling (28° to 30° C) during bypass. In contrast, Haeffner-Cavaillon and coworkers¹⁵ failed to detect TNF after various cardiac operations, which, in view of the temperature-dependency of cytokine production, could be explained by the colder temperatures (26.7° C) that prevailed during bypass in their patient population. The low levels of IL-1 β seen in the present study are also in keeping with those previously

reported by other investigators,^{15,16} but may actually underestimate the true magnitude of IL-1 production because of the presence of circulating IL-1 inhibitors.¹⁵ In contrast, the IL-6 response to CPB was strong and sustained over the 24 initial hours after operation, and although the highest IL-6 levels were again observed in the normothermic group, substantial amounts of this cytokine were yet detected in hypothermically perfused patients. Similar findings have been made by Butler and coworkers¹⁶ and are consistent with the role of IL-6 as an important mediator of the acute-phase response to injury.

The vasodilatory effects of cytokines are illustrated by our finding that, regardless of the bypass temperature, cytokine levels were significantly higher in the subgroup of patients who required pressor support, because of vasodilation-induced hypotension, than in the subgroup that did not. Also consistent with our data on cytokine production is the observation that the incidence of this vasopressor support was twofold higher in normothermic patients than in their hypothermic counterparts. We acknowledge that other factors than elevated cytokine levels can cause vasodilation during or after bypass. In this study, however, efforts were made to minimize the confounding effect of the most important of these factors. Thus anesthetic management was uniform in all patients throughout the study period. Likewise, the two groups were comparable with regard to hematocrit values. Thus, taken together, both our experimental and clinical observations strongly suggest that peripheral vasodilation associated with warm heart operation results from an enhancement at normothermia of the whole-body inflammatory response to CPB¹² and more specifically of its cytokine-mediated vasodilatory component. This hypothesis is to some extent supported by the opposite observation that induction of hypothermia during bypass reduces both complement activation and the cellular responses to this activation.²⁷

Practical implications. Blockade of cytokine production can be achieved by clinically available drugs like glucocorticoids or pentoxifylline,¹⁸ which have been clinically investigated in the treatment of vasodilation-induced hypotension occurring in the context of septic shock or heart operations.¹³ However, in view of the preceding considerations, a simpler means of addressing this issue of vasodilation could be to allow the core temperature to drift spontaneously during bypass (usually down to 32° to 34° C) and to keep giving cardioplegia at the same temperature as that of the systemic perfusate. Our recent clinical experience suggests that this "tepid" heart surgery should allow maintenance of myocardial aerobiosis

during arrest while blunting most of the vasodilatory response to strictly normothermic bypass.

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