Acute Systemic Inflammation Impairs Endothelium-Dependent Dilatation in Humans

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Background—We tested the hypothesis that endothelial dysfunction underlies the association between an acute inflammatory episode and the transiently increased risk of a cardiovascular event by examining the effects of an experimental inflammatory stimulus on endothelium-dependent vasodilation.

Methods and Results—Salmonella typhi vaccine was used to generate a systemic inflammatory response in healthy volunteers. In 12 subjects, dilatation of the brachial artery to flow and to sublingual nitroglycerin (NTG) was recorded (conduit vessel response), and in 6 subjects, venous occlusion plethysmography was used to measure forearm blood flow during intrabrachial infusion of the endothelium-dependent dilators acetylcholine (ACh) and bradykinin (BK) and the endothelium-independent dilators NTG and verapamil (resistance vessel response). Responses were assessed 16 hours before and 8 and 32 hours after vaccination. Vaccination resulted in elevations in white cell count and serum levels of interleukin-6 and interleukin-1 receptor antagonist. Eight hours after vaccination, resistance vessel responses to BK (P=0.0099) and ACh (P=0.0414) were markedly attenuated, and brachial artery flow-mediated dilatation was depressed. Resistance vessel responses to verapamil and NTG were unchanged, as was the conduit vessel response to NTG. Thirty-two hours after vaccination, resistance vessel responses to BK and ACh had returned to normal.

Conclusions—S typhi vaccine generates a mild inflammatory reaction associated with temporary but profound dysfunction of the arterial endothelium in both resistance and conduit vessels to both physical and pharmacological dilator stimuli. This finding might explain the association between infection and inflammation and the enhanced risk of an acute cardiovascular event. (Circulation. 2000;102:994-999.)

Key words: endothelium ▪ nitric oxide ▪ coronary disease

Epidemiological and observational studies have suggested an association between infection or inflammation and risk of cardiovascular disease.1–8 Two patterns of association have emerged: a link between chronic low-grade inflammation/infection and the slow process of atherogenesis,2–4 and an association between an acute systemic inflammatory response and a transiently increased risk of an acute cardiovascular event.5–8 The latter may underlie the increased cardiovascular morbidity and mortality seen after respiratory tract infection,9 severe illness requiring intensive care;6 or surgery.10

Previously, we have suggested that changes in endothelial activity may underpin the link between inflammation and the risk of an acute cardiovascular event.11 Loss of the normal vasodilator, antiplatelet, and anti thrombotic properties of the vascular endothelium might tip the balance in favor of vasospasm, thrombosis, and inflammation and may contribute to the transition between “stable” and “unstable” atheroma. We have shown that local administration of certain proinflammatory cytokines impairs endothelium-dependent dilatation in human veins in vivo.12 In this study, we test directly the hypothesis that a mild systemic inflammatory response (generated by the administration of a vaccine) impairs endothelium-dependent dilatation in the arterial circulation.

Methods

Subjects
The protocol was approved by the University College London Hospitals Research Ethics Committee. All clinical studies were performed in a temperature-controlled laboratory (24°C to 26°C). Male and female subjects (age, 22 to 37 years) who stated that they were healthy, were not taking any medication, and had not received typhoid vaccination in the previous 6 months were included.

Generation of an Inflammatory Response
Salmonella typhi capsular polysaccharide vaccine 0.025 mg (Typhim Vi, Pasteur Merieux MSD) was injected into the gluteus muscle at 8
AM on the morning of day 2 of the study. In 6 subjects, the time course of the inflammatory response was documented by measurement of body temperature (by mercury thermometer) and pulse and blood pressure (by an automated device; Dinamap, Critikon Inc) immediately before and hourly for 8 hours after administration of the vaccine. At each of these time points, blood samples were taken for measurement of white cell and platelet counts and of cytokines. A further sample was also taken 32 hours after vaccination.

Measurement of Cytokines
Serum, obtained by centrifugation, was placed in aliquots and stored at −70°C for the measurement of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interleukin-1 receptor antagonist (IL-1Ra) with a commercially available ELISA (Quantikine human IL-1β, IL-6, TNF, and IL-1Ra immunoassays, R&D Systems).

Assessment of Forearm Blood Flow (Resistance Vessel Response)
Mercury-in-Silastic strain-gauge plethysmography was used to measure forearm blood flow (mL/100 mL forearm per minute) in both arms as described previously.13,14 For each study, the brachial artery of the nondominant arm was cannulated with a 27-gauge needle (Cooper’s Needle Works) inserted under local anesthesia (2 mL of 1% lignocaine). Drugs or normal saline (sodium chloride 0.9% wt/vol) were infused continuously at 0.5 mL/min. During recording periods, the hands were excluded from the circulation by inflation of wrist cuffs to 200 mm Hg. Forearm blood flow responses to intrabrachial infusions of 4 vasodilator drugs—bradykinin (BK), acetylcholine (ACh), nitroglycerin (NTG), and verapamil—were measured on 3 occasions at 4 PM on 3 consecutive days: 16 hours before and 8 hours after vaccine administration. The needle was removed at the end of each study period. Measurement of basal blood flow was made over a 15-minute period before drug infusion. The order of drug infusions was varied between studies, but because of its long duration of action,15 verapamil was always infused last. Saline was infused for 15 minutes between each drug infusion, and blood flow recordings were then made for a further 2 minutes to ensure that flow had returned to baseline values before the next drug infusion. The ratio of flow in the infused/noninfused (control) arm was calculated for each measurement period. Vasodilator responses were expressed as the percentage increase in the ratio of forearm blood flow (infused/noninfused drug) relative to the immediately preceding baseline flow, as described previously.13,15 In a further study of 5 control subjects, forearm blood flow responses to the 4 dilator drugs were compared 24 hours apart (at 4 PM) to determine whether there was a vaccine-independent change in dilator responsiveness over time.

Measurement of Brachial Artery Dilatation in Response to Flow and NTG (Conduit Vessel Response)
Brachial arterial diameter in the nondominant arm was measured with high-resolution external vascular ultrasound (Acuson 128XP/10 with a 7.0-MHz linear-array transducer). The vessel was scanned in longitudinal section, and the center was identified when the clearest views of the anterior and posterior artery walls had been obtained. Images were magnified with a resolution box function and gated with the R wave of the ECG. End-diastolic images of the artery were acquired every 3 seconds with customized data-acquisition software (Information Integrity) and stored in digital format offline for later analysis.16 Arterial diameter over a 1- to 2-cm segment was determined for each image with a semiautomatic edge-detection algorithm. Blood flow velocity in the brachial artery was recorded continuously throughout the study with pulsed-wave Doppler. Brachial artery diameter and blood flow velocity were measured continuously for 1 minute at baseline, during 5 minutes of reduced blood flow (induced by inflation to 300 mm Hg of a pneumatic cuff placed at a site distal to the segment of artery being analyzed), and for a further 5 minutes during reactive hyperemia after cuff release.

Drug Infusions
BK was obtained from Clinalfa AG; NTG, from David Bull Laboratories; ACh, from Sigma Chemical Co; and verapamil, from Knoll Ltd. BK, NTG, and ACh were prepared as stock solutions that were placed in aliquots and stored at −20°C until use. A fresh vial of verapamil was used for each study. Cumulative dose-response curves were constructed to BK (20, 40, and 80 pmol/min, each dose for 3 minutes), ACh (25, 50, and 100 nmol/min, each dose for 3 minutes), NTG (4, 8, and 16 nmol/min, each dose for 3 minutes), and verapamil (20, 40, and 80 nmol/min, each dose for 3 minutes).

Statistical Analysis
Results are expressed as mean ± SEM unless otherwise stated. For conduit vessel responses, FMD and NTG dilatation were compared at each time point by a paired t test. For resistance vessel studies, dose–forearm blood flow response curves were constructed for all 4 drugs at each of the time points, and comparisons were made by repeated-measures ANOVA or 1- or 2-way ANOVA as appropriate. The paired t test or 1-sample t test was used for assessment of changes in inflammatory indexes and cytokine levels. P < 0.05 was considered statistically significant.

Results
Systemic Response to Vaccination
In the 8 hours after administration of capsular polysaccharide typhoid vaccine, total white cell count rose from 4.0 ± 0.3 × 10^9/L at baseline to 6.1 ± 0.5 × 10^9/L at 8 hours (P < 0.01) (see the Table). There was a progressive rise in the serum level of IL-6 from 2.1 ± 0.4 pg/mL before vaccine to 5.8 ± 3.2 pg/mL 8 hours after vaccine (P = 0.07), and the level of IL-1Ra rose from a baseline value of 188.0 ± 35.9 pg/mL to peak at 593.6 ± 198.0 pg/mL 3 hours after vaccination, a 191% increase from baseline (P < 0.05). There was no rise in the plasma level of IL-1 or TNF-α (data not shown) at the time points studied. Two subjects experienced local discomfort at the injection site, and 2 subjects reported generalized myalgia and headache. Temperature did not change significantly: 36.5 ± 0.06°C at baseline and 36.7 ± 0.03°C at 8 hours (P = NS).

Resistance Vessel Responses
Vaccine Study
Vaccination had no effect on blood pressure or resting heart rate, nor did it alter baseline forearm blood flow measured by venous occlusion plethysmography. Mean baseline blood flow was 4.72 ± 0.64 mL/100 mL forearm per minute before vaccination, 4.70 ± 0.70 mL/100 mL forearm per minute 8 hours after vaccination, and 4.04 ± 0.54 mL/100 mL forearm per minute 32 hours after vaccination (P = NS). Before vaccination, all subjects showed a dose-dependent increase in forearm blood flow to the endothelium-dependent vasodilators BK and ACh (Figure 1a and 1b) and to the endothelium-independent vasodilators NTG and verapamil (Figure 1c and 1d). When the dilator response was reassessed 8 hours after return to baseline, vessel diameter was again measured continuously for 5 minutes after administration of 50 µg of sublingual NTG. Flow-mediated dilatation (FMD) was defined as the maximum percentage increase in vessel diameter during reactive hyperemia: NTG-mediated dilatation was defined as the maximum percentage increase in vessel diameter after sublingual NTG. In 12 subjects, measurements of FMD and NTG dilation were made serially 16 hours before and 8 hours after vaccination.
after vaccination, there was a selective and marked blunting of the response to BK (P<0.0099 by repeated-measures ANOVA; Figure 1a) and an impaired response to ACh (P=0.0414 by repeated measures ANOVA; Figure 1b). Impairment of the ACh response was most marked at the highest dose of ACh used (P=0.0004 by repeated-measures ANOVA of the 100-nmol/min data). There was no significant change in the response to NTG (Figure 1c) or to verapamil (Figure 1d). Thirty-two hours after vaccination, the response to BK had returned to normal (Figure 1a), and the responses to NTG (Figure 1c) and verapamil (Figure 1d) were again unchanged. The endothelium appeared more sensitive to the effects of ACh at this time point, at least at the 25- and 50-nmol/min doses, although the response to the maximum dose of ACh was similar to the level observed before vaccination.

Control Study
When forearm blood flow studies were performed twice (24 hours apart) in 5 nonvaccinated subjects, the responses to BK (Figure 2a), ACh (Figure 2b), and NTG (Figure 2c) were unchanged over the 24-hour period. There was a significant reduction in the response to verapamil on day 2 (P=0.0093 by 2-way ANOVA; Figure 2d), although individual dilator responses to this drug proved to be more variable than for the other agents.

Conduit Artery Responses
Baseline brachial artery diameter and blood flow velocity–time index did not change during the study. However, compared with prevaccination values, there was a significant impairment in FMD by 8 hours after vaccination (mean FMD, 6.5±0.5 before versus 5.0±0.5 at 8 hours after vaccination; P<0.05; Figure 3). In contrast, there was no difference in the NTG response at these 2 time points (9.5±0.7 versus 9.5±0.9, P=NS; Figure 3).

Discussion
Intramuscular injection of capsular polysaccharide typhoid vaccine produced a mild systemic inflammatory response in healthy volunteers that was associated with profound, but temporary, suppression of endothelium-dependent relaxation in the forearm circulation. These findings demonstrate that even a relatively mild systemic inflammatory response is associated with significant alteration in endothelial function of a type commonly thought to be associated with increased cardiovascular risk.13,17–19

Inflammatory Response to Vaccination
To initiate a systemic inflammatory response, we gave an intramuscular injection of the capsular polysaccharide typhoid vaccine Typhim Vi.20 In the 8 hours after vaccination, there was a mild leukocytosis but no change in blood pressure or heart rate (see Table). Over this time, the plasma concentration of the proinflammatory cytokine IL-6 increased, but there was no change in the concentration of either IL-1β or TNF-α. These results are similar to those reported previously with the whole-cell typhoid vaccine21 and suggest that IL-6 may be an important cytokine contributing to the response seen after this type of vaccination.

Although the serum concentrations of IL-1β and TNF-α did not change, the concentration of the endogenous IL-1Ra was elevated at 3 hours after vaccination. IL-1Ra is usually synthesized in response to IL-1β generation,22 and the results are compatible with the suggestion23 that significant local cellular or tissue generation of cytokines can occur without an increase in circulating concentrations. Therefore, we cannot exclude the possibility that IL-1β and TNF-α were synthesized after vaccination or indeed that other cytokines that we did not measure contributed to the responses seen. However, in relation to cardiovascular risk, the elevation of IL-6 is of particular interest because IL-6 is presumed to be an important, if not the principal, stimulus to the synthesis of C-reactive protein,24 and elevated C-reactive protein seems to be predictive of risk of cardiovascular events.2,4

The increase in IL-6 and IL-Ra levels observed in vaccinated subjects cannot be explained by diurnal changes in these cytokines. IL-1Ra shows no diurnal variation,25 and although the levels of IL-6 do vary over a 24-hour period in subjects with inflammatory disorders,26 the levels are highest in the morning and not in the afternoon, as we observed in the vaccinated subjects.

Endothelium-Dependent Responses After Vaccination

Resistance Vessel Studies
Forearm arteriolar vasodilation was assessed in healthy volunteers before and at various times after vaccination, with
each study performed at the same time of day. Dilator dose-response curves were constructed to local intra-arterial infusion of agents whose dilator action is dependent on a functional vascular endothelium (BK and ACh) and to agents whose action is to relax directly vascular smooth muscle (NTG and verapamil). BK and ACh have been used widely to probe the ability of the endothelium to generate vasodilator factors; both agents work in part through stimulation of nitric oxide generation in this vascular bed. Decreased efficacy or potency of BK or ACh in the forearm arterial bed has been detected in patients with a wide variety of cardiovascular disease states, including hypertension, diabetes, and hypercholesterolemia, and has been taken as an indication of endothelial dysfunction. In the present study, responses to BK 8 hours after vaccination were suppressed by $65\%$. The response to ACh was also diminished, particularly at the highest dose used. This finding of selective impairment in ACh responses at doses equivalent to $\geq 100 \text{ nmol/min}$ has also been noted in patients with hypertension. Suppression of endothelium-dependent dilation was not seen in nonvaccinated individuals studied on consecutive days, and the magnitude of change seen after vaccination was as at least as great as the changes reported in the presence of classical cardiovascular risk factors. The effect of vaccination was specific for agonists working through the endothelium, because responses to the nitric oxide donor drug NTG and the calcium channel blocker verapamil were unaltered. In the case of BK, the defect in endothelium-dependent relaxation had returned to normal by 32 hours. At this time point, the endothelium appeared more sensitive to the lower doses of ACh, although the response to the maximal dose of ACh was unchanged. The reason for this difference between the 2 agonists with respect to the recovery of endothelium dependent dilator function is not clear. It may reflect differences in the recovery of receptor or postreceptor signaling mechanisms or might be the result of greater interindividual variability in the response to ACh, which has been documented previously. Indeed, the data for ACh were found to be particularly variable, and the results with this agonist should be interpreted with caution.

In nonvaccinated subjects, the responses to BK, ACh, and NTG were unchanged over 24 hours. The dilator response to verapamil was depressed on day 2 of the study in these subjects. The more marked intersubject variability in the response to verapamil might account for this observation, but in any case, it was clear that vaccination did not suppress the dilator response to this agent. In summary, for resistance vessels, vaccination caused a clear suppression of BK dilation without altering the response to NTG. The response to ACh was also altered, but these results should be interpreted in light of a greater individual variability in ACh response. Verapamil response was unchanged by vaccination, but again the responses to this agent are more variable.

**Conduit Vessel Studies**

After vaccination, the attenuation in endothelium-dependent vasodilatation in resistance vessels in response to pharmacological stimuli was mirrored by an attenuation in the dilator response of a conduit vessel to a physical stimulus (flow). This attenuation in response occurred in the absence of any...
change in the response to a submaximal dilator dose of sublingual NTG. FMD of a peripheral artery appears to be dependent on the release of endothelium-derived nitric oxide, and a reduction in brachial artery FMD has been observed in individuals with risk factors for atherosclerosis, including hypercholesterolemia, and in those with established coronary artery disease. Therefore, an attenuation in brachial artery FMD has also come to be regarded as indicating the presence of endothelial dysfunction.

The results demonstrate that vaccination with capsular polysaccharide typhoid vaccine temporarily but profoundly impairs the ability of the arterial endothelium to produce endogenous vasodilators in response to agonist and physical stimuli. The endothelium is an important transducer of physical and chemical signals from the lumen of the vessel, and experiments in animals and in vitro suggest that the changes reported here could alter vascular behavior to contribute to disruption of tissue oxygenation, increased platelet and white cell adhesion to the vessel wall, and a predisposition to vasospasm. Relaxation to BK was affected most, and this may be particularly important because local generation of BK plays a role in vascular homeostasis.

Mechanisms and Clinical Implications

The mechanism(s) by which inflammation may impair endothelium-dependent relaxation are not fully understood. One possibility is that certain cytokines induce de novo expression of the inducible isoform of nitric oxide synthase (iNOS) in the vessel wall (an isoform implicated in the high-output nitric oxide production seen in inflammation and sepsis), and this high output of NO, coupled with the generation of superoxide, causes endothelial damage. This is unlikely to be the explanation of our findings because expression of iNOS would be expected to cause vasodilatation and neither blood pressure nor resting forearm flow changed significantly after vaccination. Furthermore, although we did not test basal nitric oxide–mediated dilatation in this study, it has been shown previously that the conversion of $^{15}$N-arginine to $^{15}$NO$_2$ (a breakdown product of NO) does not increase after administration of whole-cell typhoid vaccine to healthy volunteers.

An alternative possibility is that the cytokines caused a decrease in the expression of the constitutive endothelial NOS (eNOS). However, this is unlikely to be the sole mechanism,
because in our previous studies in human veins we found no decrease in mRNA encoding eNOS after cytokine administration and because, in the earlier study, the defect was not confined to nitric oxide–mediated dilatation but also included prostanoid-mediated dilatation. Further studies are required to elucidate the mechanisms underlying the effects seen and the extent to which the endothelium loses its capacity to respond to other chemical and physical signals after inflammation.

Systemic inflammation far more severe and long-lasting than the insult produced by vaccination occurs in a wide variety of infective disorders and after iatrogenic procedures such as abdominal surgery. There is growing evidence that acute systemic inflammation is associated with an increase in the risk of cardiovascular events that may persist for days or weeks.8,9 There is also evidence that unstable angina is associated with inflammation, which might precede the onset of the syndrome. The present study demonstrates that even a mild inflammatory reaction disturbs endothelial regulation of vascular tone in the arterial circulation. It is important to determine whether the changes reported here are also seen after clinical inflammatory states and whether they might be a target for therapy.

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