Inflammatory Response After Influenza Vaccination in Men With and Without Carotid Artery Disease

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Objective—Inflammatory markers are associated with vascular disease; however, variation in the acute phase response (APR) has not been evaluated. We evaluated whether the APR magnitude in men with severe carotid artery disease (CAAD) (>80% stenosis) differed from that of men without stenosis (<15% stenosis).

Methods and Results—White males with (n = 43) and without (n = 61) severe CAAD receiving clinical influenza vaccinations were recruited. Their baseline and 24-hour after vaccination blood samples were assayed for C-reactive protein (CRP), IL-6, and serum amyloid A (SAA). In vivo APR to vaccination was measurable and varied among subjects. Adjusted for age, smoking, oral hypoglycemics, aspirin, and stain use, the relative 24-hour changes in levels of ln(CRP), ln(IL-6), and ln(SAA) were higher in men with CAAD than in men without, but only the SAA response was significant (P = 0.02); the relative SAA response was 1.6 (95% confidence interval, 1.1 to 2.5) times higher in men with than without CAAD. The APR for all markers appeared to be independent of baseline levels.

Conclusions—Influenza vaccination results in a mild, but measurable, APR in men with and without CAAD. SAA APR variability may be a predictor of severe vascular disease that is independent of basal SAA level. (Arterioscler Thromb Vasc Biol. 2006;26:2738-2744.)

Key Words: acute phase response ■ inflammation ■ carotid artery disease

Inflammatory mechanisms play a key role in the initiation and progression of cardiovascular disease.1 Increased levels of inflammatory markers such as fibrinogen, C-reactive protein (CRP), serum amyloid A (SAA), and IL-6 are associated with an increased risk of cardiovascular disease.2-5 These proteins are also key components of the acute phase response (APR) to injury or infection. After an insult, the acute phase response is characterized by a rapid increase in levels of inflammatory cytokines and acute phase proteins6 that regulate immune response, mediate inflammation, transport products generated during the inflammatory process, or participate in tissue repair and remodeling. The APR is largely regulated by the nuclear factor kappa beta (NFKB) pathway,7,8 which is activated systemically by cytokines.

To date, most studies of inflammation and cardiovascular disease risk have focused on chronic, basal levels of inflammatory markers, but the total burden of inflammation, both chronic and the acute response, may also be etiologically relevant for the development of cardiovascular disease.9 We recently detected genetic variation in the NFKB1 locus that predicted carotid artery disease status, suggesting a role of APR in disease etiology (Carlson et al, submitted, Human Genetics). Additionally, it has been reported that common acute infections are associated with an increased risk of myocardial infarction10,11 and stroke,11,12 presumably when the APR has been activated. A high degree of inter-individual variation in the acute phase response has been described by Verschuur et al, who assessed changes in fibrinogen, CRP, and IL-6 in 25 healthy volunteers after yellow fever vaccination.9 This variation suggests possible genetic and other determinants of APR, which may predispose to vascular disease.

We are unaware of previous studies that test whether APR predicts or varies with vascular disease status. To address this issue, we investigated the APR in white men with severe carotid artery disease (CAAD) and similarly aged men without vascular disease. Using influenza vaccination as a standard immune stimulus, we measured the association between CAAD disease status and change in plasma levels of inflammatory markers (CRP, IL-6, SAA) after vaccination.

Methods

Study Population

White males aged 38 to 88 years who planned to receive clinical influenza vaccinations during October to December of 2005 were...
plasma samples. The intensity of the scattered light in the nephelometers specific to CRP are agglutinated when mixed with CRP in the Nephelometer. Polystyrene particles coated with monoclonal anti-

highly sensitive method using Dade Behring reagent on a Behring II

reduce measurement variability.

points for each subject were assayed on the same plate to further

elsewhere.13

Further CLEAR study recruitment details are reported

CONTROL study. Controls were more likely to enroll in this sub-study

in our sub-study were healthier, more mobile, and more likely to seek

is a convenience sample of the CLEAR study population. Because

recruited from the PSVAHCS and UWMC patient population, but

vascular disease by patient report or medical record review and

recruited from CLEAR without severe stenosis had no previous

stenosis unilaterally or bilaterally on ultrasound, or previous

declining at 4 hours after vaccination) and current use of systemic

assays were blinded to case status. CRP and SAA

exclusions were blinded to case status. CRP and SAA

were also currently using statins and aspirin. Rather

CRP levels of subjects with baseline CRP

Laboratory Methods

Inflammation and lipid measures were performed by the North-

Laboratory Methods

Statistical Analysis

Inflammatory marker levels were neutral log (ln)-transformed for

Baseline levels for men with and without CAAD were

compared using 2-sided t tests with P<0.05 as the threshold for

statistical significance, as were 24-hour levels. For each inflamma-
tory marker, a relative change variable was calculated as the ln

(24-hour, level/baseline level), which is equivalent to ln(level at 24

hours) − ln(level at baseline). This variable defined the “response.” In

all linear regression models, the “response” or change in marker

levels between the 2 time points, ln(level at 24 hours/baseline level) was

the outcome.

In univariate analyses in the controls, we investigated the associa-
tion between response and the following potential confounders: age,

body mass index, current smoking, HDL cholesterol levels, chronic

camorbidities and use of aspirin, anti-inflammation medication, and

statins. Multivariable linear regression models with robust standard

errors were used to test for differences in the response variables

between men with and without CAAD.

Using Bland-Altman plots,18 in which the mean of 2 measure-

tments is plotted on the x-axis and the difference between the 2

measurements is plotted on the y-axis, we investigated whether

variation in the 24-hour level was dependent on the magnitude of the

mean level. We further tested the independence of baseline SAA and

the SAA response in the prediction of APR using logistic regression

models. Specifically, we tested whether the addition of the response

variable to models with and without a baseline level variable

improved prediction of CAAD status. We also report the increase in

risk of severe CAAD associated with higher SAA baseline level and

with a higher SAA response (both in terms of a 1-ln increase). Data

were analyzed using STATA (v.8.2) statistical software (StataCorp,

College Station, Tex, 2005).

Results

Traditional cardiovascular risk factors such as advanced age

and current cigarette smoking were significantly higher in

men with CAAD than in men without (Table 1). Men with

CAAD were also more likely than men without CAAD to be

currently using statins (84 versus 26%, p<0.001) and aspirin (61 versus

33%), which may suppress the APR.

CRP levels of subjects with baseline CRP ≥16 mg/dL

(range, 17.4 to 41.7) were lower at 4 hours, suggesting a

pre-vaccination insult that began resolving at 4 hours, thus

supporting exclusion of those subjects because of acute

illness. Two other subjects with CRP >10 mg/dL (15.7 and

10.9) had a 4-hour change in CRP that fell on the

regression line with the remaining subjects and, thus, were

not excluded. As reported in the literature, insulin has an

anti-inflammatory effect,19,20 which we also observed for

the 2 men with CAAD and 1 man without CAAD using

insulin in our study. They had a significantly reduced APR

and were also currently using statins and aspirin. Rather

than estimate an adjustment for insulin use based on only

3 data points, we excluded the 3 men from our analyses.

Excluded subjects are not shown in Table 1 and all

exclusions were blinded to case status. CRP and SAA

responses were expected to be maximal at 24 hours and
had changed little by 4 hours. IL-6 levels were also higher at 24 hours than at 4 hours, thus, the baseline to 24-hour response was considered for all 3 markers.

Baseline levels of CRP and SAA were slightly higher in men with CAAD than in men without, but the differences were not statistically significant (Figure 1). IL-6 followed a similar pattern (not shown). Similarly, 24-hour postvaccination levels of IL-6, CRP, and SAA were also higher in men with versus without CAAD, but only SAA was significantly higher, \( P = 0.021 \). The postvaccination levels of all markers were significantly higher than baseline levels for both men with CAAD (\( P = 0.009 \) for each marker) and for men without CAAD (\( P = 0.014 \) for each marker). In terms of percent difference (rather than ln-transformed level), the percent difference (SD) in SAA after vaccination was 222.7% (1120.9) in men with CAAD and 33.8% (101.4) in men without CAAD. For CRP and IL-6, respectively, men with CAAD had percent difference (standard deviation [SD]) values of 227.0% (995.9) and 40.1% (113.4) compared with 63.1% (125.5) and 14.0% (34.5) in men without CAAD.

To investigate potential confounders, we examined the association between the potential confounders and APR response in men without CAAD in univariate models. Of the characteristics listed in Table 1, only age was significantly associated with APR; age predicted SAA response (\( P = 0.001 \)) and CRP response (\( P = 0.029 \)) in men without CAAD, with increased age being associated with a slightly reduced APR. Other variables that appeared to have a strong effect on the APR in men without CAAD, regardless of their statistical significance, were included in our model only if they were also associated with CAAD status. Smoking, oral hypoglycemics, statin, and aspirin use were carried forward in the analyses because of reported associations with inflammation. Unless otherwise stated, adjustment was made for age (con-
TABLE 2. Linear Regression Results for the Relative Difference in APR Associated With CAAD Status

<table>
<thead>
<tr>
<th>Model Adjustment</th>
<th>exp(CAAD)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln(SAA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age*</td>
<td>1.34</td>
<td>1.02–1.75</td>
<td>0.039</td>
</tr>
<tr>
<td>Age, current smoking, aspirin use, and oral hypoglycemic use</td>
<td>1.62</td>
<td>1.03–2.55</td>
<td>0.039</td>
</tr>
<tr>
<td>Age, current smoking, aspirin, oral hypoglycemic, and statin use</td>
<td>1.63</td>
<td>1.08–2.48</td>
<td>0.022</td>
</tr>
<tr>
<td>Ln(CRP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.19</td>
<td>0.90–1.58</td>
<td>0.236</td>
</tr>
<tr>
<td>Age, current smoking, aspirin use, and oral hypoglycemic use</td>
<td>1.40</td>
<td>0.88–2.21</td>
<td>0.158</td>
</tr>
<tr>
<td>Age, current smoking, aspirin, oral hypoglycemic, and statin use</td>
<td>1.30</td>
<td>0.84–2.02</td>
<td>0.240</td>
</tr>
<tr>
<td>Ln(IL-6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.13</td>
<td>0.97–1.33</td>
<td>0.124</td>
</tr>
<tr>
<td>Age, current smoking, aspirin and oral hypoglycemic use</td>
<td>1.14</td>
<td>0.92–1.43</td>
<td>0.237</td>
</tr>
<tr>
<td>Age, current smoking, aspirin, oral hypoglycemic, and statin use</td>
<td>1.07</td>
<td>0.86–1.35</td>
<td>0.546</td>
</tr>
</tbody>
</table>

*Covariate was statistically significant in the model, P<0.05.

exp(CAAD), the regression coefficient, is interpretable as the fold increase in response for men with CAAD vs men without CAAD.

tinuous), current cigarette smoking (yes/no), aspirin use on day of vaccination (yes/no), and statin and oral hypoglycemics use (yes/no). The use of aspirin on the day of vaccine was highly correlated with chronic aspirin use (r^2 = 0.73). Therefore, we believe adjustment for use of aspirin on the day of blood draw to be appropriate. Current use of NSAIDS was much less common (prevalence <10%) than aspirin use in both groups of men.

For all inflammatory markers, men with CAAD had larger responses than men without CAAD (Table 2). Responses were greatest for SAA; men with CAAD had a 1.6-times higher response than men without CAAD after adjustment for all confounders (95% confidence interval [CI], 1.1 to 2.5). For CRP, men with CAAD had a 1.3-times higher response than men without (95% CI, 0.8 to 2.0). Only the SAA response was significantly higher in men with versus without CAAD, both before (P = 0.039) and after adjustment for potential confounders (P = 0.022). Of those subjects using aspirin, most were on low-dose aspirin (80 mg/d), which is not expected to have a sustained anti-inflammatory effect. When 15 men (10 cases and 5 controls) on high-dose aspirin (≥300 mg/d) were excluded from analyses, we found slightly higher and more significant responses for all markers in men with versus without CAAD. Three men received a second vaccine (Pneumovax) at the time of flu vaccination. Exclusion of these men, with (n = 1) and without CAAD (n = 2), from the regression models did not significantly affect results.

The Bland-Altman plots (Figure 2) use the mean of the baseline and 24-hour response instead of the baseline to reduce spurious patterns related to consideration of baseline when plotted against the difference. They demonstrate several points. First, the overall mean response, as indicated on the y-axis by the mean line, and the distribution of the individual responses about the mean and zero response (no change) lines can be seen. Second, cases generally appear to have higher responses than controls. Finally, the plots did not indicate that response (difference between baseline and 24-hour measurements) was related to the mean values, because the points appeared to be scattered evenly around the mean line for the range of observed mean values. This result implies that the response measure was not dependent on level. Indeed, there is no positive correlation between response and baseline level (data not shown). A similar pattern was observed for IL-6 (not shown).

To further assess their independence as predictors of case status, SAA response and baseline SAA were jointly considered in an adjusted logistic model. In this joint analysis SAA response remained significant, P = 0.009, supporting independence of the APR from baseline level for CAAD prediction. For each one ln unit increase in SAA response, the odds of having severe CAAD were 24.6 (95% CI, 2.5 to 268.7). The SAA response was also more strongly associated with CAAD status than baseline SAA level in separate models; the OR associated with CAAD status for a one ln unit increase in baseline SAA was 1.9 (95% CI, 0.5 to 6.8), whereas for a one ln unit increase in SAA response it was 4.4 (95% CI, 1.2 to 17.1).

Discussion

In this study, we took advantage of the seasonal influenza vaccination to investigate whether in vivo vaccine response was predicted by vascular disease status. Our results are in agreement with previous studies showing that vaccination does induce a mild acute immune response. However, previous studies were conducted in small numbers of healthy volunteers. Our study is unique in contrasting the APR among diseased and healthy men. We observed a larger inflammatory response in men with extant severe CAAD than in men without severe CAAD. Although our study was limited in size, we found a consistently increased response in men with versus without CAAD across different inflammation markers, but only
SAA was significantly higher in men with CAAD. Normal plasma levels of SAA are estimated to be approximately 3 mg/L in individuals without chronic inflammatory conditions but may increase to $\geq 1000$ mg/L within 24 to 48 hours of an acute stimulus. Our results suggest that the magnitude of SAA response after flu vaccination is approximately 60% higher in men with versus without severe CAAD. Although the degree of immune response may depend on vaccine formulation, all participants in this study received the same dose of inactivated flu vaccine.

Because of study logistics, we may have failed to measure IL-6 at its peak. We observed a larger change in IL-6 at 24 than 4 hours (data not shown). Tsai et al found a significant increase in IL-6 1 day after flu vaccination in healthy individuals, but did not serially measure for the peak. In a study of cytokine levels after surgery in middle-aged subjects, IL-6 was found to peak after 4 to 6 hours, whereas in a small study of yellow fever vaccination, daily measures of IL-6 indicated a peak at 5 days after vaccination. Because of the large individual variability in IL-6, we may have needed additional measurements to best characterize the IL-6 response.

Previous research has reported increased levels of inflammatory markers during convalescence, after surgery, and during stroke events. In particular, research on stroke victims has suggested that levels of CRP after a stroke event may predict severity and recurrence of future events within 1 year, independent of other risk factors. While these results may be caused by pre-event elevations or post-event responses, our results suggest that APR measurement may provide different information about the burden of inflammation than measurement of chronic levels of inflammatory markers. Using Bland-Altman plots, we did not see evidence that the magnitude of change during the acute phase response was dependent on level of the marker. Baseline levels, although higher in men with CAAD, were not positively correlated with response, which suggests that the magnitude of APR, if a vascular disease risk factor, may predict vascular disease independent of baseline levels of inflammatory markers. Using logistic regression models in which we assessed the associations between CAAD status and the SAA response and baseline level independently and together in the same model, we provided additional evidence that the APR variable is associated with CAAD status independently of baseline SAA level.

Men with previous severe stenosis were very homogeneous with respect to disease severity and the majority were taking statins and low-dose aspirin. Both statins (3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitors) and aspirin have anti-inflammatory effects, with aspirin known to impede the NFkB response. Statins, which are commonly prescribed as lipid-lowering agents, have also been reported to lower CRP levels independent of their effects on lipids. In contrast to the anti-platelet clotting effect, which persists until the turnover of platelets (~7 days), the anti-inflammatory effects of aspirin are largely transient, although in one controlled trial, high doses (300 mg/d) of aspirin were reported to modestly decrease levels of IL-6 and CRP after 6 weeks of treatment. Most subjects in our study were on low-dose aspirin. When 15 men on high-dose aspirin were excluded from the analysis, we found slightly higher and more significant responses in men with CAAD than men without for all markers. We were unable to do a similar sensitivity analysis excluding men on statins because of the high prevalence of use in men with CAAD. Inclusion of the 3 subjects on insulin did not influence the results.
April Rodenbaugh and Laura McKinstry for their technical assistance.

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Disclosures

None.

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