last acute event. Such condition is associated with an enhanced responsiveness to proinflammatory stimuli. Thus, NF-κB activation might represent a mechanism by which CRP amplies and perpetuates the inflammatory component of acute coronary syndromes.  

10:45 a.m.

852-2 Widespread Coronary Inflammation in Patients With Unstable Angina

Antonio Bittar, Luiz M. Bissacuti, Giovanna Luzzo, Gianluca Comerio, Giuseppe D'Oriofilo, Filippo Crea, Attilio Maseri, Catholic University, Rome, Italy.

Background: Inflammation may cause unstable angina (UA) by favoring coronary plaque rupture and erosion. In UA activated leukocytes were found in peripheral and coronary sinus blood, but it is unclear whether they are selectively activated in the vascular bed of the culprit lesion. Methods: We measured the difference in neutrophil myeloperoxidase content (MPX) across the cardiac and femoral circulation in 5 groups of patients: 33 with UA and single stenosis either in left descending (LAD) artery (UA-L, 24 patients) or right coronary artery (UA-R, 10 patients), 13 with stable angina, 13 with variant angina and 6 controls. Blood samples were taken simultaneously from aorta, femoral vein and cardiac vein (GCV) which selectively drains LAD but not right coronary blood (as we confirmed by the presence or absence of evidence of vasodilation in the GCV following nitroglycerin injection into left and right coronary arteries respectively). Plasma levels of C-reactive protein (CRP) were also measured.

Results: Aortic MPX was similar in UA-L and UA-R (-3.9 and -5.5), but significantly lower in the other three groups (all p<0.05). A significant negative correlation was found between aortic MPX and CRP values (r=-0.45, P=0.025). MPX was significantly decreased in GCV both in UA-L (-6.4, P=0.001) and UA-R (-6.6, P=0.003), but not in stable angina with LAD stenosis, variant angina with LAD ischemia, or controls. No MPX decrease was observed in any group in the femoral vein. The GCV-aorta gradient was also significantly correlated with CRP levels (r=-0.41, P=0.011).

Conclusions: The widespread inflammatory activation of neutrophils across the coronary vascular bed, independent of culprit stenosis location and of ischemia, carries important pathophysiological implications and challenges the concept of a single coronary vulnerable plaque in unstable coronary syndromes.

11:00 a.m.

852-3 Apoptosis Resistance of Neutrophils in Patients With Unstable Angina

Luigi M. Bissacuti, Annalisa Forment, Claudia Colussi, Beatrice Merlino, Francesca Recondo, Laura Morlacchi, Francesca Giganti, Giovanna Luzzo, Attilio Maseri, Cardiology, Catholic University, Rome, Italy.

Background: Activated neutrophils have been found in the peripheral and coronary circulation of pts with unstable angina (UA). As apoptosis limits neutrophils pro-inflammatory functions, we hypothesized that a prolonged survival of peripheral neutrophils could represent an important inflammatory mechanism leading to instability.

Methods: We investigated spontaneous apoptosis of circulating neutrophils in 31 pts with Braunwald class IIIB UA, 12 pts with stable angina (SA) and 32 normal subjects (N). Peripheral blood samples were taken within 12 hours from the last ischemic episode; neutrophils were isolated by gradient centrifugation (Polymorprap, N/100ml) and cultured at 37°C. Spontaneous apoptosis of neutrophils was analyzed by flow cytometry using Annexin V-FITC (Immunotech, Marseille, France) at 4, 20 and 48 hours after isolation. CRP was also measured by a high-sensitivity method (Dede-Behering).

Results: Data are reported as median (range). The apoptotic rate was significantly lower in UA vs SA and N at 4 hours: the percentage of apoptotic neutrophils was 11% (2-21) in UA, 45% (6-75) in SA and 29% (13-62) in N (p<0.01, UA vs SA and N). The apoptotic delay persisted in UA pts at 20 and 48 hours, being at 20 hours 72% (40-94) in UA, 76% (29-95) in SA, 83% (91-96) in N and at 48 hours 84% (39-98) in UA, 87% (87-97) in SA and 95% (47-100) in N (p<0.05 UA vs N). The apoptotic rate was significantly lower in pts with UA-R vs UA-L and SA (p<0.01). For the patients with values >500 ng/ml, neutrophil apoptosis was not different in N vs UA pts.

Conclusions: Our study demonstrates a significant neutrophil apoptosis resistance in pts with UA. A prolonged survival of peripheral neutrophils suggests that an enhanced proinflammatory potential of these cells may represent a novel mechanism of instability.

11:15 a.m.

852-4 C-Reactive Protein-Mediated Complement Activation During Acute Myocardial Infarction In Humans


Background: Complement activation during acute myocardial infarction (AMI) may contribute to a worse course, and is a potential target for therapy. The mechanism of complement activation during AMI is still unclear, but likely involves C-reactive protein (CRP), as this acute phase protein is present in infarcted human myocardium together with activated complement fragments, and CRP was found to increase infarct size in a rat model of AMI via complement activation. We, therefore, examined CRP-mediated complement activation in blood samples of humans with AMI or unstable angina pectoris (UAP).

Methods: CRP-complement complexes (CRP-C3b and CRP-C4d), specific markers for CRP-dependent complement activation, were measured in serial plasma samples of patients with AMI (n=82) or UAP (n=25). Peak levels and cumulative generation of the complexes in time were related to those of complement activation fragments (C3a, C3b/C4b, and C3b/C4b/C5b-9) in patients with AMI than in patients with UAP. The 48-hours cumulative release of CRP and generation of CRP-C4d complexes correlated significantly with enzymatic infarct size, and was most pronounced in AMI patients without reperfusion therapy. Complement fragments, CRP and CRP-complement complex levels did not differ between patients with or without adverse events during 1-year follow-up, but were found to be associated with these adverse events, when using a merit value (= sensitivity * specificity - 100%) of >50%.

Conclusions: Peak levels and cumulative generation of complement activation fragments during the 48 hours-observation period were significantly higher in patients with AMI than in those with UAP. Complement activation during the first 12 hours was significantly higher in AMI patients compared to thrombolysis, than in patients treated with direct angioplasty. The generation of CRP-complement complexes was also significantly higher in patients with AMI than in patients with UAP. The 48-hour cumulative release of CRP and generation of CRP-C4d complexes correlated significantly with enzymatic infarct size, and was most pronounced in AMI patients without reperfusion therapy. Complement fragments, CRP and CRP-complement complex levels did not differ between patients with or without adverse events during 1-year follow-up, but were found to be associated with these adverse events, when using a merit value (= sensitivity * specificity - 100%) of >50%.

11:30 a.m.

852-5 sPLA2 Potentiates Binding of CRP to Ischemically Challenged Cardiomyocytes

Remco Niemeijer, Hans W. Niessen, Cees A. Visser, Yvonne P. Lubbers, Caspar G. Schalkwijk, Erik C. Hack, Free University Medical Hospital (VU), Amsterdam, The Netherlands.

Introduction: Myocardial infarction induces a systemic and local inflammatory response. Recently we found co-localising depositions of C-reactive protein (CRP) and secretory Phospholipase A2 (sPLA2) in human infarcted myocardium; sPLA2 occupying a larger area than CRP. We hypothesise that sPLA2 may be involved in the formation of suitable ligands for binding of CRP to infarcted cardiomyocytes. We have studied this in adult rabbit cardiomyocytes and H9C2 cells.

Materials: Adult rabbit cardiomyocytes were isolated by collagenase perfusion of the heart. H9C2 cells were cultured according to ATCC guidelines. CRP was isolated from human sera. While sPLA2 was isolated from HEPG2 cells by means of affinity chromatography. The activity of sPLA2 was validated. Ischemia was mimicked by means of metabolic buffer. Subsequent to ischemia cardiomyocytes were reperfused with or without sPLA2 and/or CRP. Cell vitality was evaluated using Propidium Iodide (PI) and Annexin-V (AV) staining. Binding of CRP or sPLA2 to the cells was evaluated by means of fluorescence immunohistochemistry.

Results: After exposure of the cells to metabolic inhibition a rapid decline in cell vitality evolved. Both in adult cardiomyocytes and H9C2 cells sPLA2 positivity was found in AV and PI double positive cells (thus late apoptotic or necrotic cells). Interestingly sPLA2 positivity was also found in single AV positive cells (thus early apoptotic or 'floating but viable cells'). H9C2 cells exposed to metabolic inhibition showed increased capacity to bind sPLA2 and CRP when exposed for a period of two hours to culture medium containing these molecules. The binding of CRP in the metabolic inhibited group was mainly to 'floating' (non-viable) cells in H9C2, while in adult cardiomyocytes previously exposed to sPLA2 the binding of CRP was significantly higher when compared to the cells not pre-incubated with sPLA2.

Conclusions: These findings support the notion that sPLA2 binds to viable but ischemic cardiomyocytes. Our findings therefore are in line with our hypothesis that inflammatory mediators increase the area of infarction subsequent to myocardial infarction.

11:45 a.m.

852-6 C-Reactive Protein Elevation Following Chemically Induced Septal Infarction In Humans Without Coronary Atherosclerosis

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Background: C-reactive protein (CRP) elevation predicts major adverse cardiac events in healthy individuals, patients with stable angina pectoris, patients with acute coronary syndromes, and in patients undergoing percutaneous and surgical revascularization. Percutaneous transluminal septal ablation in hypertrophic cardiomyopathy (HCM) allows for the elimination of the CRP risk factor, which is considered to be due to myocardial infarction (MI) without the confounding influence of an unstable coronary plaque.

Methods: Eleven patients with HCM and without angiographic evidence of coronary artery atherosclerosis undergoing septal ablation were studied. Myocardial infarction was induced by infusing alcohol into septal perforating arteries. Blood was obtained at baseline and at 48 hours. High sensitivity CRP analysis was carried out by immunonephelometry, using an Immage analyzer (Beckman Coulter).

Results: Baseline CRP values were available on 7 patients. Mean CRP at baseline was 1.1 ± 0.9 mg/dl in patients with values of CRP <1 mg/dl had a median CRP 322A ABSTRACTS- Myocardial Ischemia and Infarction