LECTURE

ΔΙΑΛΕΞΗ

Hemostasis and inflammation in cardiovascular risk

Emerging concepts from genetic epidemiology

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Αιμόσταση και φλεγμονή στον καρδιαγγειακό κίνδυνο. Αναδυόμενες αντιλήψεις από τη γενετική επιδημιολογία

Περίληψη στο τέλος του άρθρου

Arterial thrombosis events, such as acute myocardial infarction (AMI), have a complex, multifactorial pathogenesis. Among the factors which may lead to activation of hemostatic components and thrombogenesis, inflammatory mediators have been recently recognized. On one hand, besides platelets, blood leukocytes appear increasingly important in atherosclerosis and thrombosis; on the other hand the levels of acute phase reactants (such as C-reactive protein) and inflammatory cytokines may influence remarkably clotting/fibrinolysis components and are associated with increased risk of AMI. The levels of inflammatory mediators (as well as of clotting factors) may be genetically modulated. Recent experience from our group has indicated that some polymorphisms in the genes of the cytokine system, able to influence the inflammatory response of monocytes, are associated with the risk of juvenile myocardial infarction, while fibrinogen genotypes associated with higher fibrinogen levels mediate the response to *Helicobacter pylori* infection and enhance the risk for familial myocardial infarction. Thus, from genetic epidemiology studies, new support is provided to the links between inflammation and arterial thrombosis.

Key words

Gene/environment interactions
Gene polymorphisms
Infection
Inflammation
Myocardial infarction

1. INTRODUCTION

Inflammation may play an important role in the initiation and progression of atherothrombotic disease. Several prospective studies have indicated that elevated levels of C-reactive protein (CRP), a non specific inflammatory marker, are associated with primary and secondary occurrence of coronary heart disease.[1,2] Pathological studies have demonstrated that atherosclerotic lesions contain infiltrates of inflammatory cells and sites of plaque ruptures are associated with inflammatory components.[3] Inflammatory cytokines appear to be relevant not only to the progression of atherosclerosis, but also to the development of thrombosis. Interleukin 1-β (IL-1β), a major proinflammatory cytokine with pleiotropic biological effects, is able to stimulate the synthesis of tissue factor (TF) from monocytes and endothelial cells,[4] the production of PAI-1 from endothelial cells[5] and the expression of adhesive molecules on the surface of platelets and leukocytes.[6] On the other hand, IL-1β stimulates the synthesis of IL-6, that, in turn enhances the production of fibrinogen, CRP and other inflammatory mediators, which have been associated with the risk of ischemic coronary disease.

2. THE ROLE OF INFECTIOUS AGENTS

An activation of acute phase reactants and inflammatory mediators may often be the result of chronic infection with pathogens, also known to promote atherosclerosis and coronary artery disease.

In particular, three infectious agents, *Chlamydia pneumoniae* (Cp), Cytomegalovirus (CMV) and Herpes simplex virus, detected within human atherosclerotic tissues, have gained considerable interest as potential pathogens causing clinical manifestations of atherosclerosis.[7] The

strongest seroepidemiologic and experimental evidence exists for Cp infection, an obligate intracellular pathogen causing respiratory tract infections, while results on Cytomegalovirus and Herpes infections are less consistent. A number of studies on middle aged or elderly subjects have confirmed the association between Cp and ischemic vascular disease; in the Helsinki Heart Study population, the presence of elevated antibody titres to Cp was an independent risk factor for the development of a coronary event in the following six months.[8] In some studies high levels of Cytomegalovirus antibodies were associated with clinically manifested atherosclerotic disease, particularly in patients with transplanted hearts. In symptom-free elderly men, recruited in the framework of the Zutphen Elderly Study, Cp, but not CMV or IIP infections, was predictive of coronary artery disease.[9] Finally, no association has recently been found between IgG antibodies to CMV or Herpes simplex virus I and the risk of future myocardial infarction and stroke in the Physician's Health Study.[10] We have recently shown that previous infection with *C. pneumoniae*, Cytomegalovirus or Herpes simplex virus I were all able to increase the risk of premature coronary artery disease, after adjustment for traditional coronary risk factors and socio-economic status. The combination of seropositivity to *C. pneumoniae* and elevated titres of Cytomegalovirus represented the most unfavorable immunologic profile for an increased risk of premature coronary heart disease.

As anticipated, we have also found enhanced inflammatory activation in patients with infections and particularly in those with multiple infections. Indeed, C-reactive protein was more elevated in patients with the above mentioned most unfavorable association of infections. In addition,both fibrinogen and C-reactive protein were higher in infected patients than in healthy subjects with similar levels of elevated antibody titres. This different inflammatory response to infections in patients and in controls suggests a possible interaction between infections and inflammation as a pathogenic component of the disease. In agreement with this hypothesis, our results showed a significant positive interaction between a high antibody titre to Cytomegalovirus and C-reactive protein levels with increasing risk of MI.

How chronic forms of viral or bacterial infections might promote atherosclerosis is so far unknown. It has been hypothesized that infectious agents might induce endothelial cell damage and plaque growth, rupture and thrombosis, through a direct infection of vascular cells or by promoting an inflammatory response that stimulated the synthesis of some acute phase reactants, such as fibrinogen and C-reactive protein. In addition, persistent immune activation might enhance the production of monocyte derived procoagulants, such as tissue factor, and stimulate the production of several cytokines (TNF-alpha, interleukin 1 and 2), which induce fibroblast and smooth muscle cell proliferation, platelet activation and thrombosis. Indeed, Cp or Hp infections have been reported to be associated with elevation of fibrinogen, C-reactive protein and leukocyte count, although other reports do not confirm these data.

3. PLATELET, LEUKOCYTES AND THROMBOSIS

The role of platelets in coronary thrombosis has long been recognized.[11,13] After plaque rupture, exposed collagen from the vessel wall, in addition to other mediators, induces platelet aggregation. Platelet-released substances, such as ADP and thromboxane A_2, stimulate adjacent platelets, further enhancing the process of platelet activation. Platelet membrane glycoproteins appear to play a role in the events of platelet adherence and aggregation and involve specific plasma adhesive proteins, such as von Willebrand factor and fibrinogen.

More recently, blood leukocytes too have been suggested to be involved in atherogenesis, thrombus formation and their regulation.[14] Epidemiological studies have shown evidence of a significant association between leukocyte count and incidence of coronary heart disease.[15,16] On one side monocytes/macrophages express tissue factor when stimulated by endotoxin or other inflammatory agents, thus contributing to blood clotting initiation;[17] on the other side, macrophages, T-lymphocytes and mast cells, infiltrating coronary lesions, may help to bring about plaque instability.[14] Additionally, polymorphonuclear (PMN) leukocyte-released substances, such as cathepsin G and elastase, may activate platelets and degrade endothelial barrier function.[18,20] It thus appears that, rather than platelets alone, platelet/leukocyte interactions could represent a crucial step in thrombogenesis. Indeed, activated platelets not only facilitate further platelet accumulation and fibrin deposition at the site of vascular injury –as it was clearly established in the past– but may recruit PMN and mononuclear leukocytes into actively forming thrombus by expressing P-selectin,[21,25] a receptor mediating platelet-leukocyte binding, which has been shown to induce tissue factor in monocytes.[26] Moreover, activated platelets release chemokines, which may undergo proteolytic cleavage to form Neutrophil Activating Peptide-2 (NAP-2).[27,28]

The pathophysiological relevance of leukocytes deposited on platelets activated within circulating blood and/or adhering at the site of vascular damage is still largely unknown. However, the presence of platelet-PMN conjugates has been reported in peripheral blood of patients with unstable angina, suggesting that such cell-cell interaction triggers PMN activation in this clinical condition,[29] a finding originally reported by Mazzone et al.[30] On the other hand, Mickelson et al have found that formation of platelet-PMN conjugates following coronary angioplasty was a predictive index of acute reocclusion.[31] In patients with acute myocardial infarction increased platelet-monocyte interaction and up-regulation of Mac-1 on monocytes have recently been reported.[32]

The intriguing possibility is emerging that platelets activated at the site of an unstable atherosclerotic plaque are unable by themselves to produce a full vascular occlusion, but might be the initial trigger of a localized leukocyte-dependent inflammatory response.

4. MYOCARDIAL INFARCTION AS A GENETICALLY MODULATED DISEASE

Although the pathogenesis of myocardial infarction is multifactorial, family and twin studies suggest an important hereditary component. A family history of myocardial infarction predicts the development of the disease in other family members. In the Italian population the frequency of relatives with myocardial infarction before the 65th year of age is 35% in patients with myocardial infarction and 20% in healthy controls.[33] Studies in twins indicate a greater genetic risk in monozygotic as compared with dizygotic twins and adoption studies have shown that much of the excess risk is genetic rather than environmental.[34] The relevance of genetic factors in determining the risk of disease can also be supposed by studying the behaviour of migrating populations.[35] There are, indeed, populations such as the Inuit, that have maintained their low risk of myocardial infarction, in spite of the adaptation to western life style, suggesting that genetic factors are important. However, this apparent inheritability of the disease cannot be considered as a disorder with a mendelian transmission; indeed, rather than segregating, myocardial infarction does aggregate in families in a way compatible with its multifactorial pathogenesis.

Polymorphisms have been identified in genes encoding for factors related to the disease development with a relatively high frequency in normal populations, that allowed to make direct association between genetic variations and development of myocardial infarction.[36] However, polymorphisms are not the cause of the disease, but their presence may increase or decrease the susceptibility to the development of the disease.

5. GENETIC REGULATION OF RISK FACTORS

Polymorphisms mainly act contributing to the variability of protein levels and activity in blood. Rather than simply influencing the basal levels of the corresponding proteins, they do modulate their individual response to environmental factors such as diet, smoking, physical activity, susceptibility to drug effects. Coagulation factor VII levels are strongly correlated with dietary fat intake and non-fasting triglyceride levels. Such correlation can be modulated by F VII genotype;[37] indeed, it has been observed in homozygotes for the RR genotype, but not in carriers of the Q allele. The effect of IIVR4 and -323 0/10 bp polymorphic loci of factor VII gene, moreover, are gender-dependent showing an effect in males but not in females.[38] These data suggest a role for gender specific factors in the regulation of FVII expression. Furthermore, gene-gene interactions could be of fundamental importance in the regulation of factor VII levels. Recently, in a study of a normal Italian population, we found a significant relation between the HVR4 locus and R353Q or the promoter -323 0/10 bp polymorphisms in modulating FVII levels. Essentially, the rare Q353 or -323 10 bp alleles are only associated with lowered FVII levels in the presence of the H7 allele of the HVR4 polymorphism.

A polymorphism in fibrinogen gene has been associated with the risk of familial myocardial infarction.[39] Carriers of the rare allele, related to the high levels of fibrinogen in blood, increased their risk of disease by about two times. In this case gene/environment interactions appear important, since the rare allele of fibrinogen ß-chain polymorphism multiplies the detrimental effect of a previous *Helicobacter pylori* infection on the risk of myocardial infarction, by an additive effect on fibrinogen levels.[40]

The effect of polymorphisms, even in the risk of multifactorial disease, implies the existence of an inherited pattern and should be more expressed in peculiar situations, like in subjects with family history or during young age. Juvenile myocardial infarction in subjects with a family history of coronary thrombosis, therefore, represents a homogeneous subset of patients in which the impact of major genes on the development of the disease can be resonably hypothesized. Familial hypercholesterolemia, a monogenic disease, due to mutations of the LDL-cholesterol receptor, has been already identified as a cause of premature myocardial infarction.

Not only clotting factors (see Factor VII) but also inflammatory mediators may be genetically modulated. IL-1ß gene lies in a cluster with other interleukin genes on human chromosome 2q 13. Several polymorphisms in interleukin genes have been described and have been related with the risk to develop inflammatory diseases. Some of these polymorphisms may contribute to the inter-individual variation in cytokine production rates upon disease. A neutral polymorphism at position +3953 of IL-lß gene has been correlated with IL-lß protein production from LPS-stimulated monocytes, the rare T-allele being associated with high levels of IL-Iß.[41] Also, C/T single base variations in the IL-1 promoter at position -511 have been described.[42] They are at almost total linkage disequilibrium between themselves and in a weak negative linkage disequilibrium with the +3953 polymorphism. Finally, a polymorphism of the interleukin-1 receptor antagonist gene has been associated with the risk of single-vessel coronary disease in an adult UK population with coronary artery disease.[43] The same polymorphism did not show different frequency in patients with juvenile myocardial infarction.[44] On the other hand, we have studied the association of polymorphisms at position -511 and +3953 of IL-lß gene with the risk of myocardial infarction at young age. 158 patients with myocardial infarction before the age of 45 (males) or 50 years (females) were frequency-matched for age and sex with 153 healthy controls selected from the general population.

Subjects carrying CC genotype of -511 promoter polymorphism showed a 3 times increase in the risk of myocardial infarction (OR=3.01; 95% CI: 1.31–6.92), after adjustment for conventional risk factors. Odds ratios increased with the genotypes in the following order: TT (OR=1) <CT<CC in univariate (P=0.006) and multivariate (P<0.0001) analysis. Multivariate linear regression evidenced a signicant association between the -511 CC genotype and the levels of fibrinogen both in cases and in controls. Subjects with the C allele showed higher levels of fibrinogen as compared with TT homozygotes. No association was found between +3953 of IL-1ß gene and the risk of myocardial infarction.[45]

6. CONCLUSIONS

Evidence is accumulating to support the concept that inflammatory mediators can initiate both atherosclerosis and thrombosis through the activation of clotting and the contribution of both platelets and leukocytes activities. The levels of inflammatory mediators and clotting factors may be genetically modulated and their polymorphisms are associated with different susceptibility to environmental factors (such as, in infectious agents). Genetic epidemiology of cardiovascular risk factors provides useful information on these issues.

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ΠΕΡΙΛΗΨΗ

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Αναδυόμενες αντιλήψεις από τη γενετική επιδημιολογία

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Τα επεισόδια αρτηριακής θρόμβωσης, όπως το οξύ έμφραγμα του μυοκαρδίου (ΟΕΜ), έχουν σύνθετη πολυπαραγοντική παθογένεια. Μεταξύ των παραγόντων που οδηγούν σε ενεργοποίηση των αιμοστατικών στοιχείων και θρομβογένεση, έχουν αναγνωριστεί οι μεσολαβητές της φλεγμονής. Από τη μία, εκτός από τα αιμοπετάλια, τα λευκοκύτταρα φαίνονται όλο και περισσότερο σημαντικά στην αθηροσκλήρυνση και θρόμβωση· από την άλλη, τα επίπεδα των αντιδρώντων της οξείας φάσης (όπως της C-αντιδρώσας πρωτεΐνης) και των κυτοκινών της φλεγμονής επηρεάζουν σημαντικά στοιχεία της πήξης/ινωδόλυσης και συνδέονται με αυξημένο κίνδυνο ΟΕΜ. Τα επίπεδα των μεσολαβητών της φλεγμονής (καθώς και των παραγόντων της πήξης) μπορούν

να τροποποιηθούν γενετικά. Σύμφωνα με την πρόσφατη πείρα της ομάδας μας, κάποιοι πολυμορφισμοί των γονιδίων του συστήματος των κυτοκινών, με δυνατότητες επηρεασμού των φλεγμονωδών απαντήσεων των μονοκυττάρων, συνδέονται με κίνδυνο νεανικού εμφράγματος του μυοκαρδίου, ενώ οι γονότυποι του ινωδογόνου που συνδέονται με αύξηση των επιπέδων του ινωδογόνου είναι μεσολαβητές της απάντησης στη λοίμωξη με ελικοβακτηρίδιο του πυλωρού και αυξάνουν τον κίνδυνο για οικογενές έμφραγμα του μυοκαρδίου. Έτσι, από γενετικές επιδημιολογικές μελέτες προκύπτουν νέες ενδείξεις, που στηρίζουν τη σχέση μεταξύ φλεγμονής και θρόμβωσης.

Λέξεις ευρετηρίου: Αλληλεπιδράσεις γονιδίων/περιβάλλοντος, Έμφραγμα μυοκαρδίου, Λοίμωξη, Πολυμορφισμός γονιδίων, Φλεγμονή

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