Leukocytosis, Vascular Disease, and Adenine Nucleotide Metabolism

To the Editor:

We read with interest the paper by Barry S. Coller on leukocytosis and its relationship with vascular disease morbidity and mortality1 and the subsequent correspondence.2,3 We are intrigued to learn that relative leukocytopenia may be associated with increased morbidity and mortality in patients with acute myocardial infarction1,4 and in those undergoing percutaneous coronary intervention.5 We note the discussion on whether leukocyte count is merely a marker of general disturbances in inflammation and general poor health, or whether leukocytes might contribute directly to thrombosis and atherosclerosis.

Although several mechanisms through which leukocytes may contribute to thrombosis and atherosclerosis were discussed,1–3 nothing has been said about the role of leukocytes in adenine nucleotide metabolism. Adenosine diphosphate (ADP) is, of course, a major contributor to the thrombotic mechanism as evidenced by the successful use of ADP antagonists which reduce ADP-induced platelet activation and aggregation and thereby act as anti-thrombotic agents.6

It was recognized that leukocytes are active in the metabolism of adenine nucleotides many years ago7 but recent papers from our own group have re-emphasized their importance.8–10 In summary, first we found that adenosine triphosphate (ATP) added to blood induces platelet aggregation via a mechanism involving leukocytes and ADP formation.8 We then found that leukocytes (all neutrophils, all monocytes and a subset of lymphocytes) test positive for an antibody to CD39, the NTPDase which converts ATP into ADP and also ADP into adenosine monophosphate (AMP).9 We also saw that leukocytosis results in modified platelet aggregation responses to both ATP and ADP; platelet aggregation in blood induced by ATP is more rapid in leukocytosis while aggregation induced by ADP is followed by rapid disaggregation. A systematic analysis of the role of blood cells and plasma enzymes in adenine nucleotide metabolism by high-performance liquid chromatography (HPLC) was then performed.10 This confirmed that leukocytes are the principle means through which ATP and ADP added to blood are broken down to ADP and AMP respectively. Thus leukocytes provide a means of metabolising adenine nucleotides that is additional to that provided by vascular endothelial cells and thus provide a clearance mechanism that is active within the blood itself as well as at the blood periphery.

In view of the current discussion on leukocytosis we show in Figure 1 the results of new experiments in which we added ATP or ADP to normal blood, blood to which autologous leukocytes had been added to increase the count from 4.4×10^3/L to 26×10^3/L and blood from a patient with hyperleukocytosis with a white cell count of 126×10^3/L. Nucleotides and products were determined by HPLC. It can be seen that in the presence of normal numbers of leukocytes ATP was converted to ADP which peaked at about 15 minutes and was then subsequently broken down to AMP; ADP was converted directly to AMP. With added leukocytes the rate of ATP metabolism is markedly enhanced (t1/2 from ~15 minutes to ~4 minutes) with ADP produced more quickly, but present for a shorter duration before conversion to AMP; the rate of ADP conversion to AMP was also markedly enhanced. In hyperleukocytosis both ATP and ADP

\[ \text{Figure 1. Metabolism of ATP (a, c and e) and ADP (b, d and f) in blood (anticoagulated with hirudin) from normal volunteers (a, b; WCC~4.4\times10^3/\mu L), blood to which autologous leukocytes had been added (c, d; WCC~26\times10^3/\mu L) and blood from a patient with hyperleukocytosis (e, f; WCC~126\times10^3/\mu L). Measurement of ATP, ADP and AMP was by HPLC.}^{10}\text{ Results are mean±SEM, n=3 (a–d) or a single determination (e, f). ATP (100 \mu mol/L) or ADP (100 \mu mol/L, except for f where 30 \mu mol/L ADP was used) was added to blood samples which were incubated at 37°C for up to 30 minutes.}\]
were metabolized very quickly with rapid conversion to AMP which was subsequently removed.

It seems to us that differences in adenine nucleotide metabolism as determined by leukocyte count may well have relevance to thrombosis and also to hemostasis. On the one hand, leukocytes in blood provide a mechanism for platelet activation via ATP, as well as ADP. Further, because high leukocyte counts provide a means of converting ATP to ADP more rapidly, earlier platelet activation can occur. In this regard, leukocytes can be thought of as being prothrombotic. On the other hand, leukocytes also provide an effective means of removing ADP thus limiting platelet responses to this important nucleotide, possibly an important anti-hemostatic role.

We believe the extent to which the increased morbidity and mortality associated with leukocytosis may be a consequence of altered adenine nucleotide metabolism needs to be investigated further. Erythrocytes are a huge source of ATP which can be released physiologically in response to hypoxia and via cell damage. Activated platelets also release ATP as well as ADP. There is also the possibility that the increased morbidity and mortality associated with leukocytopenia in acute myocardial infarction and in percutaneous coronary intervention may be a consequence of ineffective ADP removal. In which case there would be further justification for the use of ADP antagonists in these conditions.

Stan Heptinstall
Jacqueline R. Glenn
Andrew Johnson
Bethan Myers
Ann E. White
Lian Zhao
Departments of Cardiovascular Medicine and Haematology
Queens Medical Centre, Nottingham
University of Nottingham and University Hospital NHS Trust
Nottingham, United Kingdom

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