To the Editor:

We read with interest that a second thrombin receptor, designated protease activated receptor 3 (PAR3), has been cloned from human and mouse tissues. The human and mouse PAR3 cDNAs were cloned by screening a human small intestinal cDNA library and a mouse spleen cDNA library, respectively. The investigators have shown the presence of PAR3 mRNA in a range of human and mouse tissues including bone marrow, by Western blot analysis, and they have demonstrated the occurrence of PAR3-specific mRNA in mouse megakaryocytes by in situ hybridization. However, the investigators do not provide any direct evidence for expression of this novel thrombin receptor in human platelets or megakaryocytes.

By using reverse-transcription polymerase chain reaction (RT-PCR) and PCR-based library screening strategies we have successfully cloned and sequenced PAR3 cDNA from human platelets and from a number of human erythroleukemic (HEL, K562) and promonocytic (U937, HL60) cell cDNA libraries.

Whole blood was collected from healthy human volunteers by antecubital venepuncture into acid citrate dextrose (final concentration, 11 mmol/L) and platelets purified by density centrifugation (Nycozem; Sigma-Aldrich Co Ltd, Poole, UK). Platelet RNA was extracted by a caesium chloride step gradient method. Platelet cDNA was synthesized from 1 μg of total RNA using oligo (dT)15 primed reverse transcription in a total volume of 25 μL (200 U M-MLV RT; 25 U rNasin ribonuclease inhibitor; 2 mmol/L dNTPs; 50 mmol/L Tris-HCl, pH 8.3; 75 mmol/L KCl; 3 mmol/L MgCl2; 10 mmol/L DTT; Promega). Aliquots of platelet, HEL, K562, U937, and HL60 cDNA were taken for PCR amplification of PAR3 cDNA using primers, as follows: forward TTCCTC-ACCTGCTGATCA and reverse ACTGGTCTCAAGCAACTATT (1.25 U Taq DNA polymerase; 0.2 mmol/L dNTPs; 1.5 mmol/L MgCl2; 20 mmol/L Tris-HCl, pH 8.4; 50 mmol/L KCl; Promega Ltd, Southampton, UK). Cycling parameters were as follows: 3 minutes at 94°C; followed by 35 cycles of 45 seconds at 94°C, 30 seconds at 55°C, 60 seconds at 72°C, and finally a 10-minute extension step at 72°C. After thermocycling, 5 μL of each 50-μL RT-PCR or PCR mixture was electrophoresed on a 1.5% agarose gel containing 10 μg/mL ethidium bromide and visualized under UV transillumination (Fig 1). A single 521-bp product was amplified by both RT-PCR from platelet total RNA and by PCR from the four cDNA libraries. Platelet RT-PCR, HEL cell PCR, and K562 cell PCR fragments were subcloned into pCRII.1 (Invitrogen BV, NV Leek, The Netherlands) and sequenced on both strands (Vista Thermosequenase; Amersham International plc, Little Chalfont, UK). Sequences obtained were compared to those held in Genbank and EMBL using the GCG program BLAST, which returned 100% sequence identity with human PAR3.

These results provide evidence for the expression of a second thrombin receptor on human platelets and on a number of erythro- and promonocytic cell lines, suggesting that PAR3 is not only found on platelets and megakaryocytes but also on cells of the monocytic lineage. Whether PAR3 acts as a functional thrombin receptor on these cell types remains to be shown.

From searches of Genbank and EMBL sequence databases, a number of other sequences were found to share approximately 45% homology with PAR3, such as PAR1, PAR2 and the P2Y1, P2Y4, and P2Y8 purinoceptors. The original aim of these cloning experiments was to identify the platelet P2T purinoceptor by amplifying P2Y purinoceptor-like sequences that may be present in a platelet mRNA population. The degenerate oligonucleotide primers used were designed from conserved regions within the putative third and seventh transmembrane domains P2Y1 and P2Y2. The degree of sequence homology with the P2Y1 purinoceptor suggests that these receptor families may be closely related and it would be interesting to see whether this novel proteinase receptor can also be activated by purines or their synthetic analogues.

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All cDNA libraries used were synthesized from oligo(dT) primed cDNA and nondirectionally cloned into the vector pCDM8 (Invitrogen). We thank Prof Janet Allen for the HEL cell cDNA library, and the UK Human Genome Mapping Project for providing the K562, U937, and HL60 cDNA libraries.
Should Patients With Atherosclerosis or Peripheral Vascular Disease Be Stratified for Factor V Leiden?

To the Editor:

We read with interest the article of Rosendaal et al1 relating to the association between factor V Leiden and myocardial infarction in young women. We were impressed by the association between smoking and the manifestation of factor V Leiden in thrombosis/atherosclerosis and the high prevalence of the heterozygote gene state in the group. We recently found a similarly high prevalence of factor V Leiden in peripheral vascular disease patients.2

Thirty-eight peripheral vascular disease patients who had undergone infrainguinal vein graft bypass were studied to determine the relationship between vein graft stenosis and hyperhomocysteinaemia (mean age, 66.8 years). Twelve patients were current smokers and 21 were past smokers, confirmed by carbon monoxide measurement. A strong association between hyperhomocysteinaemia and vein graft stenosis was found, especially in men.3 In these patients and seven others who underwent infrainguinal bypass grafting with prosthetic material we performed a detailed hematological analysis including activated protein C resistance (APCR). We were surprised to find 8 of 33 patients (24%) not taking Warfarin had APCR. To better define this population, polymerase chain reaction (PCR) for factor V Leiden was performed on all 45 patients and it revealed 6 patients with APCR to be factor V Leiden–positive. PCR showed that 1 patient on Warfarin therapy and 1 patient without APCR were factor V Leiden–positive. The prevalence of factor V Leiden in this cohort was 18% (8 of 45), compared with a locally determined prevalence of 3.5%—similar to published United Kingdom prevalences. Of the 8 patients with factor V Leiden, 1 patient was homozgyous. There was no difference in the prevalence of hyperhomocysteinaemia in patients with (5 of 8) or without (17 of 38) factor V Leiden; however, the one homozygous factor V Leiden patient had the highest homocysteine level that we measured at 45 μmol/L. He had no history of deep venous thrombosis. Of the 8 patients with factor V Leiden, all except 1 were past or present smokers.

We would suggest that there is an association between hyperhomocysteinaemia, factor V Leiden, smoking, and atherosclerosis as well as deep venous thrombosis but that the interaction between cause and effect is not clear. The exciting possibility of predicting not only thrombotic risk but the risk of atherosclerosis in patients with recognized risk factors might exist if the manifestation of these genetic factors and their relationship with environmental factors was better understood. We believe that the opportunity to risk stratify all patients with atherosclerosis with respect not only to recognized risk factors such as smoking but also systemic factors such as factor V Leiden now exists and would allow a more accurate assessment of the need for therapeutic intervention.

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REFERENCES

Epstein-Barr Virus Lytic Infection in Lymphocytes and the Persistence of the Virus

To the Editor:

In their interesting report, Prang et al1 showed the presence of peripheral blood mononuclear lymphocytes (PBMC) positive for Epstein-Barr virus (EBV) lytic proteins. This is in agreement with the updated theory of EBV persistence.2 However, this is not the first paper reporting lymphocytes expressing EBV lytic proteins in vivo.

It used to be believed that epithelial cells are responsible for the primary infection and the persistence of EBV. EBV lytic infection in epithelial cells is differentiation-associated and virus released there infects lymphocytes later on.3 The first report proposing a different view for the role of EBV-infected lymphocytes in the persistence of the virus was published in 1988.4 Later in 1990, Rickinson4 proposed a new model which shows how lymphocytes can contribute to the persistence of the virus through expansion at the germinal center, and also to the transmission of the virus to distant epithelial cells by spontaneous lytic infection. Rickinson’s model still emphasizes the role of epithelium in the primary infection of EBV. In 1994, Niedobitek and Young5 proposed that both primary EBV infection and the persistence of the virus are mediated by lymphocytes. How can mucosal lymphocytes become infected by EBV without the help of epithelial cells if the epithelium is not damaged? The presence of intraepithelial lymphocytes may provide an explanation. I have previously shown the presence of EBV-infected intra-epithelial lymphocytes in the nasopharyngeal mucosa of EBV seropositive people who had no EBV-associated disease or malignancy6 and the
Cloning of PAR3 cDNA From Human Platelets, and Human Erythroleukemic and Human Promonocytic Cell Lines

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