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# Transient Intermittent Lymphocyte Activation Is Responsible for the Instability of Angina

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**Background.** Blood clotting activation is an important component of the inflammatory response; the outbursts of unstable angina are usually associated with increased thrombin formation and coronary mural thrombosis.

**Methods and Results.** To investigate 1) whether monocyte activation is responsible for the enhanced thrombin formation during bursts of unstable angina and 2) what mechanism(s) might be responsible for monocyte activation, we studied patients with unstable angina ( $n=31$ ), stable effort angina ( $n=23$ ), left endoventricular thrombosis ( $n=8$ ), and control subjects ( $n=44$ ), measuring plasma fibrinopeptide A (FPA) levels and the capacity of monocytes to express procoagulant activity (PCA) and of lymphocytes to modulate this expression. Patients with unstable angina and patients with endoventricular thrombosis had significantly ( $p<0.0001$ ) higher FPA plasma levels than patients with effort angina and control subjects. However, only monocytes from unstable angina patients expressed significantly increased PCA characterized as tissue factor-like activity (units/ $10^5$  monocytes, median and range; 120, 1.1–463.2 versus 10.8, 0.8–39.1 in control subjects;  $p<0.0001$  versus the other groups). When 14 patients with unstable angina were restudied 8–12 weeks later, they showed neither elevated plasma FPA levels nor monocyte PCA. In unstable angina patients, there was a correlation between FPA and PCA ( $r=0.56$ ,  $p<0.001$ ). For expression of PCA by monocytes, both an incubation of at least 2 hours with lymphocytes and direct monocyte-lymphocyte contact were needed. In reconstitution and cross-mixing experiments, only lymphocytes from patients with active unstable angina induced the expression of PCA by monocytes from both control and patient groups.

**Conclusions.** The results demonstrate that the increased thrombin formation in unstable angina patients is due to the expression of tissue factor-like activity by activated monocytes. The monocyte activation appears to be a part of a lymphocytic cell-instructed response intermittently triggered by unknown factors. (*Circulation* 1992;86:790–797)

**KEY WORDS** • monocytes • angina, unstable • angina, effort • tissue factor

Increased thrombin generation, evaluated as raised fibrinopeptide A (FPA) plasma levels, almost invariably occurs during outbursts of unstable angina.<sup>1–3</sup> Coronary mural thrombi have been frequently detected in angiographic,<sup>4–7</sup> angiographic,<sup>8</sup> and postmortem studies.<sup>9</sup> Plaque fissuring followed by platelet activation and contact of flowing blood with local prothrombotic substrates are the most widely proposed mechanisms for the increased thrombin formation and the beginning of the acute thrombotic event of unstable angina.<sup>8–12</sup> However, doubts have arisen about plaque fissuring as a direct cause of either the increased thrombin formation or development of mural thrombosis because fissured plaques can be found in 10% of

individuals dying of noncardiac causes,<sup>13,14</sup> and, not infrequently, neither intimal lesions nor thrombi can be found in patients who died because of unstable angina.<sup>15</sup> Moreover, no beneficial clinical effects have been obtained by thrombolytic treatment of patients with unstable angina,<sup>16–20</sup> indicating that mural thrombosis does not play a major role in the pathogenesis of unstable angina. There is clear-cut evidence that activation of blood coagulation is an important component of the inflammatory response, especially during the immune response.<sup>21–25</sup> Coronary atherosclerotic plaques contain abundant lymphoplasmic cell and monocyte-macrophage infiltrates,<sup>26–29</sup> which are found much more frequently to be markedly increased into the site of plaques in subendothelium and in perivascular nerves of unstable angina patients with fatal outcome than of patients with effort angina who died of noncardiac causes, even with the same degree of coronary luminal narrowing.<sup>30–32</sup>

After *in vitro* exposure to immune or nonimmune stimuli, monocytes express tissue factor on their surfaces<sup>24,33–40</sup> and can specifically activate blood clotting.

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Monocyte-macrophage activation resulting in procoagulant activity (PCA) formation might contribute to the increased thrombin generation and, most importantly, might be a causative link between inflammation and instability of angina. The aims of the present study were to investigate 1) whether monocyte activation occurs during bursts of unstable angina, 2) whether it may be responsible for activation of clotting and enhanced thrombin formation in these patients, and 3) what mechanism(s) might be responsible for monocyte activation.

## Methods

### Study Population

We studied 31 consecutive patients aged less than 70 years with primary unstable angina (type IIB and IIIB, according to Braunwald<sup>41</sup>) and 23 patients with stable effort angina. We studied 35 apparently healthy subjects of equivalent ages with normal lifestyles and without any limitations in physical activity (healthy controls), nine patients with chest pain caused by noncardiac causes (three intercostal fibrositis, two spontaneous pneumothorax, and four motor disorders of the esophagus), and eight patients with endoventricular thrombi complicating idiopathic dilatative cardiomyopathy as control subjects. This last group represented control subjects with activated blood clotting. Unstable angina was defined as chest pain occurring at rest or on minimal effort (washing, speaking, combing) without any increase in the creatine kinase MB fraction, with ECG evidence of myocardial ischemia (transient ST segment displacement  $>0.1$  mV during chest pain), and angiographic evidence of coronary artery disease. Patients were continuously monitored by ECG for the first 3 days before blood sampling, and coronary angiography was performed after the blood specimen was drawn. Stable effort angina was defined according to the following criteria: 1) typical anginal pain on effort and no anginal attacks at rest during at least the previous 3 months, 2) no asymptomatic ischemic episodes at rest during 3-day Holter monitoring, 3) stable ischemic threshold during at least three stress exercises in the week preceding the study, and 4) angiographic evidence (at least one stenosis  $>70\%$ ) of coronary artery disease. Patients with effort angina had been on nitrates and calcium antagonists for at least 1 week. Patients were excluded from the study if they had enzymatic or ECG evidence of myocardial infarction, clinical evidence of recent infections, or were suffering from diabetes, immunological disorders, or neoplastic disease. Patients were also excluded if they had undergone surgical or invasive procedures in the month preceding the study. Patients were taking no drugs that would interfere with platelet function or blood clotting such as heparin, oral anticoagulants, or antiplatelet drugs before the blood sampling. In 10 unstable angina patients, mononuclear PCA was investigated before and during heparin administration (priming dose, 5,000 IU followed by 1,000 IU/hr<sup>42</sup>). Blood was drawn in the morning after overnight fasting and within 48 hours of the most recent episode of chest pain. Fourteen of 31 patients with unstable angina were restudied after a convalescent period of at least 8–12 weeks, at a time when they had been free of symptomatic or asymptomatic (Holter

monitoring) ischemic episodes for at least 2 weeks. Nine stable effort angina patients and nine normal control subjects were also restudied. All patients gave their informed consent to use part of their blood samples for an experimental study.

### Experimental Procedure

**Cell preparations.** Mononuclear cells were obtained from peripheral citrated blood. Briefly, platelets were removed by two centrifugations, and mononuclear cells were separated by density gradient centrifugation<sup>43</sup> and washed twice with phosphate-buffered saline-EDTA to eliminate further platelet contaminants and finally were resuspended ( $1 \times 10^7$  cells/ml) in RPMI-1640 plus gentamicin (100  $\mu\text{g/ml}$ ). Mononuclear cells were more than 98% viable by trypan blue exclusion and contained  $<2\%$  polymorphonuclear leukocytes and  $<1\%$  platelets. Cells were identified by  $\alpha$ -naphthylacetate esterase staining<sup>44</sup> and by flow cytometric analysis by using monoclonal antibodies (OKM14, OKPanB, OKT3, Ortho Diagnostic Systems, Milan, Italy). The percentage of monocytes was  $20.4 \pm 1.7\%$  in mononuclear preparations. Pure preparations (98%) of both monocytes and lymphocytes were obtained by incubating mononuclear cell suspensions on precoated Petri dishes for 1 hour.<sup>45</sup> To look for a role of lymphocytes in inducing the expression of PCA by monocytes, PCA was assayed separately in pure monocyte and pure (98%) lymphocyte preparations and in cell suspensions obtained by mixing lymphocytes from patients or control subjects with monocytes from control subjects or patients. In addition, purified monocytes were incubated in the presence of lymphocytes in a coculture system (Costar Transwell-Cal), preventing direct cell-cell contact. In the cross-mixing experiments, the relative proportion of lymphocytes to monocytes was 4:1, which has been found optimal for the collaborative effect of lymphocytes on monocytes.<sup>46</sup> We assayed the effects of supernates obtained from pure lymphocytes incubated for 4 hours. These supernates were added to pure monocyte suspensions, incubated for 4 hours, and PCA was assayed. All reagents were negative for endotoxin contamination at the level of 0.005 ng/ml (Limulus amoebocyte lysate assay, E-Toxate, Sigma Chemicals, St. Louis, Mo.).

**Assay and characterization of procoagulant activity.** Cell preparations were incubated for 0, 2, 4, 8, 12, and 18 hours at 37°C in a humidified 5% CO<sub>2</sub> atmosphere and disrupted by freezing, thawing, and sonicating. PCA of cells was assayed by one-stage plasma recalcification time<sup>47</sup> and expressed in arbitrary units (units/10<sup>5</sup> monocytes). In preliminary experiments, the PCA was characterized as tissue factor by evaluating its sensitivity to phospholipase C (Calbiochem, San Diego, Calif.),<sup>48</sup> Concanavalin A (Sigma),<sup>49</sup> and cysteine protease inhibitor (HgCl<sub>2</sub>)<sup>50</sup> and by using factor VII and factor X deficient plasmas. To rule out direct interference of heparin administered to the patients with the assay of the PCA produced by mononuclear cell suspensions, heparin was added to the assay system in specially designed experiments just before determination at a concentration of the same order of that in vivo (0.2–0.3 IU/ml final concentration). Under these experimental conditions, heparin did not influence the assay of PCA.

TABLE 1. Characteristics of the Subjects

	Healthy controls	Noncardiac chest pain	Effort angina	Unstable angina	Endoventricular thrombosis
No.	35	9	23	31	8
Men	24	6	17	22	5
Women	11	3	6	9	3
Age (years)	51.7±13.2	55.9±9.8	53.8±7.8	54.6±9.1	58.6±5.8
Weight (kg)	72.5±9.5	69.8±8.2	69.1±7.4	70.2±6.5	71.6±6.8
Cholesterolemia (mmol/l)*	4.9±0.7	5±0.8	5.2±0.7	5.1±0.6	5.3±0.6
Blood pressure (mm Hg)					
Systolic	135±13	138±12	143±15	140±16	139±11
Diastolic	82±6	80±7	80±6	79±6	81±6
Mean angiographic score	...	...	16.4±3.9	15.9±5.9	...

Values are mean±SD.

\*To convert cholesterol values to milligrams per deciliter, multiply by 38.67.

**Plasma fibrinopeptide A.** Plasma FPA assay was performed by the ELISA method according to Gaffney et al<sup>51</sup> and Soria et al,<sup>52</sup> using commercial kits kindly supplied by Boehringer Biochemia (Milan, Italy). The intra-assay and interassay coefficients of variation were 5.9% and 7.8%.

### Coronary Angiography

Coronary angiography was performed by Judkin's technique. The occurrence and severity of coronary angiographic lesions were evaluated from at least three projections by the score suggested by the American Heart Association.<sup>53</sup>

### Statistical Analyses

The analyses were performed by an IBM PS/70 computer and BMDP statistical software. Demographic data were analyzed by ANOVA. Values of FPA and PCA were initially assessed for normality by the kurtosis test. On the basis of these results, nonparametric procedures were used to compare data from the various groups. Unless otherwise indicated, results are given as medians and ranges. The nonparametric Kruskal-Wallis test for one-way ANOVA (H test) was used for the differences among the various groups; the Wilcoxon rank-sum test for unpaired and paired data was used for comparisons between individual groups. For the correlation analysis, Spearman's rank correlation coefficient was used. The PCA was calculated by regression analysis. All probability values reported are two-tailed, with values of less than 0.05 considered statistically significant.

## Results

### Clinical Characteristics

Characteristics of patients and control subjects are shown in Table 1. All patients with unstable angina had had anginal attacks and silent ischemic episodes the first day after hospital admission and were on nitrates plus calcium antagonists. The number of angina attacks over the 3-day monitoring period averaged 3.4±5.9 (mean±SEM) per patient per day, and the silent ischemic episodes averaged 7.2±5.3 per patient per day.

All patients with both unstable angina and stable effort angina had angiographic evidence of coronary artery disease (Table 1). There were no significant

differences in severity or in extent of angiographic coronary lesions between the two groups of patients (Table 1).

### Thrombin Formation

All except one patient with unstable angina showed increased thrombin generation, as revealed by elevated plasma levels of FPA (Figure 1). FPA plasma levels were also elevated in the seven patients receiving nitrates and calcium antagonists who had not had any ischemic episodes (Holter monitoring) for at least 36 hours before blood sampling. FPA plasma levels of unstable angina patients (median, 7.1 ng/ml; range, 1.5–14.1) were similar to those found in patients with intraventricular thrombosis (median, 8.2 ng/ml; range, 5.3–11.4) but significantly higher than those of control subjects (healthy control subjects: median, 1.8 ng/ml; range, 0.8–3.7 [ $p<0.0001$ ]; noncardiac chest pain patients: median, 1.7 ng/ml; range, 1.5–3.4 [ $p<0.0001$ ]) (Figure 1). Unlike the patients with unstable angina, only four of 23 patients with stable effort angina had increased levels of FPA in plasma. The FPA values of this group of patients did not differ from those of the healthy control subjects (median, 1.7 ng/ml; range, 1.5–4.6) (Figure 1). All of the 14 patients with unstable angina who were restudied 8–12 weeks later, when they were spontaneously free of myocardial ischemia, had significantly lower FPA plasma levels ( $p<0.001$ ) than their original values, at this time not differing from controls (Figure 2).

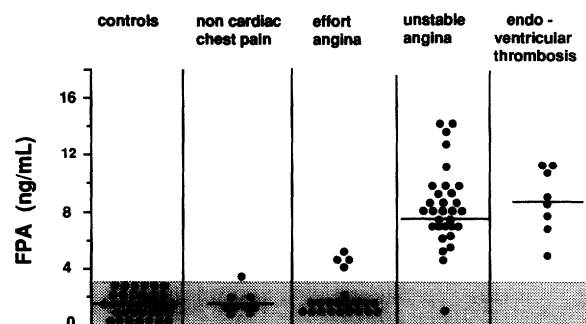


FIGURE 1. Plot of fibrinopeptide A (FPA) plasma concentrations. Lines indicate median values; shaded area indicates control range.

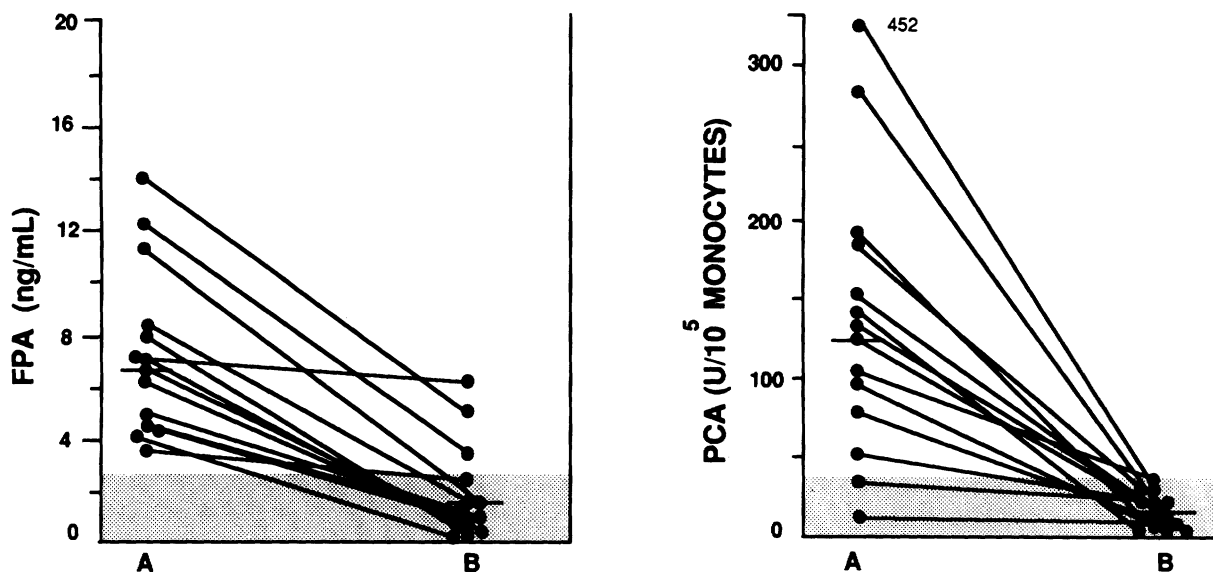


FIGURE 2. Plots show fibrinopeptide A (FPA) (left panel) and procoagulant activity (PCA) (right panel) in the same patients when suffering from myocardial ischemic episodes (A) and after 8–12 weeks when free from ischemic episodes (B). Lines indicate median values; shaded area indicates control range.

**Mononuclear Cell Procoagulant Activity**

There were no significant differences in the monocyte content of the mononuclear cell preparations from the different groups ( $F=0.13$ , NS). The time courses of the reaction leading to the formation of PCA by mononuclear cells are reported in Figure 3. In all the assays, the highest PCA was expressed after 4 hours and did not increase during the remaining 14 hours of incubation (Figure 3).

By the procedures described in “Methods,” mononuclear cell PCA was characterized as tissue factor-like activity (data not shown). Mononuclear cells evaluated immediately after separation from blood expressed only scanty amounts of PCA, without significant differences among the five groups of subjects ( $H=-1.85$ ,  $p=0.18$ ; Table 2). Conversely, after a 4-hour period of incubation, mononuclear cells from all except two patients with unstable angina showed elevated amounts of PCA (median, 120.1 units/ $10^5$  monocytes; range, 1.1–463.2 units/ $10^5$  monocytes), whereas the mononuclear cells

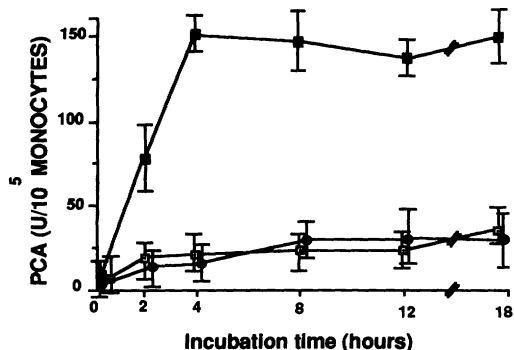


FIGURE 3. Plot shows time course of procoagulant activity (PCA) expressed by mononuclear cell preparations (bars indicate standard deviation) ( $n=10$ ). Closed squares, unstable angina patients; closed circles, effort angina patients; open squares, control subjects.

from the control subjects and patients with endoventricular thrombosis or stable effort angina produced PCA in trace amounts (Figure 4) without further increase. Mononuclear cells from the seven patients who did not have angina attacks in the 24 hours preceding blood sampling also showed elevated PCA levels (median, 128.8 units/ $10^5$  monocytes; range, 101.1–218.6 units/ $10^5$  monocytes). Pure (98%) neutrophil, lymphocyte, and monocyte preparations from unstable angina patients and from control subjects did not develop any significant PCA during an 18-hour incubation period (data not shown). In contrast to the first determinations, mononuclear cells from the 14 patients with unstable angina who were restudied 8–12 weeks later, when angina free, expressed only negligible amounts of PCA not different from that expressed by control subjects or patients with stable angina (Figure 2). In the group of patients with unstable angina investigated in both active and inactive phases, there was good correlation between FPA plasma levels and the amount of PCA produced by mononuclear cells ( $r=0.56$ ,  $p<0.001$ ) (Figure 5). Conversely, there were no significant relations between plasma FPA levels or the amount of PCA and the severity of coronary angiographic lesions ( $r=0.08$  and  $r=0.07$ , respectively, NS). The addition of plasma (1:10) from the control subjects or from angina patients to the incubation medium increased the expression of monocyte PCA without any significant difference between patients or controls (data not shown).

TABLE 2. Procoagulant Activity (Units/ $10^5$  Monocytes) Formed by Mononuclear Cells Immediately After Separation

	Healthy subjects ( $n=35$ )	Non-cardiac chest pain ( $n=9$ )	Effort angina ( $n=23$ )	Unstable angina ( $n=31$ )	Endo-ventricular thrombosis ( $n=8$ )
Median	0.28	0.29	0.30	0.33	0.30
Range	0.1–0.9	0.1–0.7	0.1–1.0	0.1–3.7	0.1–0.8

For calculating median values, the value of 0.1 was attributed to samples below the detection limit.

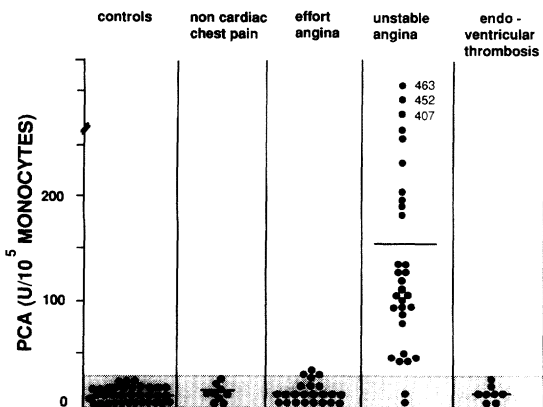


FIGURE 4. Plot shows procoagulant activity (PCA) of mononuclear cell preparations after 4-hour incubation. Lines indicate median values; shaded area indicates control range.

**Reconstitution and Cross-Mixing Experiments**

The addition of pure (98%) lymphocyte preparations from patients with unstable angina to pure (98%) monocyte preparations from control subjects or from patients with stable effort angina induced the formation of significant amounts of PCA in 4 hours of incubation (Figure 6). On the contrary, the addition of lymphocytes from control subjects or from patients with endoventricular thrombosis or from patients with stable effort angina to monocytes from control subjects or from unstable angina patients induced expression of insignificant PCA (Figure 6). Thus, only lymphocytes from patients with unstable angina were able to induce the expression of tissue factor-like activity in pure monocyte preparations. However, no significant PCA was expressed by the addition of lymphocyte supernates to monocytes or when direct monocyte-lymphocyte contact was prevented.

**Effects of Heparin on Plasma Fibrinopeptide A and Mononuclear Procoagulant Activity**

Ten patients with active unstable angina were treated by continuous intravenous heparin infusion (1,000 IU/hr). Twelve hours after the start of heparin infusion, FPA plasma levels were significantly lower in all patients but had not returned to the normal range. Simultaneously, the expression of tissue factor-like activity by mononuclear cells decreased from 129.6 units/10<sup>5</sup> monocytes (range, 46–463.2 units/10<sup>5</sup> monocytes) to 27.4 units/10<sup>5</sup> monocytes (range, 8.7–54.5 units/10<sup>5</sup> mono-

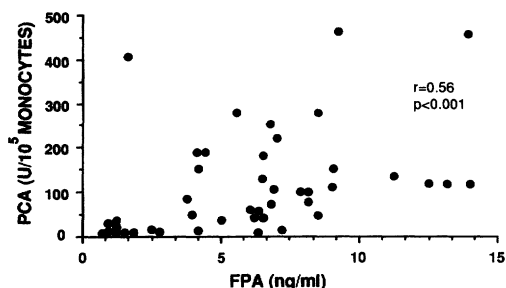


FIGURE 5. Plot shows relation between fibrinopeptide A (FPA) and procoagulant activity (PCA) in unstable angina patients investigated in both active and inactive phases.

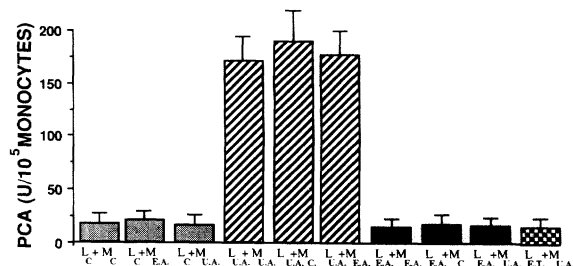


FIGURE 6. Bar graph shows effect of cellular interactions on procoagulant activity (PCA) (mean ± SD). L, lymphocytes; M, monocytes; C, controls; E.A., effort angina; E.T., endoventricular thrombosis; U.A., unstable angina; C+E.A., n=9; C+U.A., n=14; U.A.+E.A., n=9; U.A.+E.T., n=5.

cytes) after a 4-hour incubation period ( $p < 0.001$ ) (Table 3). However, heparin administration did not affect the capacity of lymphocytes to induce procoagulant expression by monocytes because pure preparations of lymphocytes from patients receiving heparin were still able to induce the expression of PCA in monocytes from control subjects (Table 3), suggesting that heparin only blocked the expression of PCA by monocytes.

**Discussion**

Our results indicate that circulating lymphocytes from patients with active unstable angina but not from control subjects or patients with stable effort angina were able to induce the expression of tissue factor activity by monocyte preparations obtained from both control subjects and patients. The lymphocyte activation and the expression of PCA by monocytes did not appear to be epiphenomena of the angina, because they were demonstrable also in patients temporarily without angina attacks because of antiangina treatment, or a consequence of thrombotic processes or blood clotting activation, because only low or very low levels of monocyte PCA were found in the monocytes from patients with endoventricular thrombosis. The lymphocyte activation

TABLE 3. Effect of Heparin Infusion ≈1,000 IU/hr for 12 Hours on Mononuclear Cell Procoagulant Activity and Fibrinopeptide A Levels

	Before heparin infusion	After heparin infusion	p
Mononuclear cell procoagulant activity (units/10 <sup>5</sup> monocytes)			
Unstable angina lymphocytes plus unstable angina monocytes (n=10)	129.6 46–463.2	27.4 8.7–54.5	<0.001
Unstable angina lymphocytes plus control monocytes (n=10)	110.9 55.8–202.5	104.4 56.3–177.2	=0.9
Control lymphocytes plus unstable angina monocytes (n=6)	15.9 12.8–34.6	15.6 12.9–33.1	=0.6
Fibrinopeptide A (ng/ml), unstable angina patients (n=10)	8.1 4.2–17.3	3.6 1.3–5.1	<0.001

Values are expressed as median and range.

and the expression of monocyte PCA appear to be distinct features of active unstable angina and not a consequence of coronary artery disease per se, because they were not detectable in the same patients restudied 8–12 weeks later, at which time they had no angina. Some evidence indicates that the lympho-monocyte activation can be responsible, at least in part, for the augmented thrombin generation in patients with unstable angina, because FPA plasma levels were elevated only in patients with mononuclear PCA formation, and there was a good relation between FPA plasma levels and the formation of tissue factor. Moreover, heparin administration simultaneously reduced FPA levels and monocyte PCA. Obviously, the monocyte mechanism does not exclude other possible mechanisms such as plaque disruption, platelet activation, or contact of flowing blood with thrombogenic substrate of the arterial wall. The results of reconstitution and mixing experiments indicate that the lymphocyte activation is the crucial moment for the PCA formation by monocytes. Thus, the activation of the lymphocytes and the elevated monocyte PCA and FPA plasma levels in patients with active unstable angina appear to be an expression of an acute transient inflammatory state, in agreement with the knowledge that activation of blood coagulation has long been recognized to be an important component of the inflammatory response, especially during the immune response.<sup>21–25</sup> Although only anatomical studies can be conclusive, the reported elevation of C-reactive protein in active coronary artery disease<sup>54</sup> and the elevated density of circulating monocyte-macrophages with major histocompatibility complex class II antigen *HLA-DR*<sup>55</sup> support this statement.

A special issue is whether monocyte activation occurs in blood circulation or in the coronary arterial wall. The formation of the PCA by monocytes appears as a response to triggered lymphocyte–monocyte contact rather than to soluble lymphokines<sup>56–58</sup> because no significant PCA was expressed when direct monocyte–lymphocyte contact was prevented or after addition of lymphocyte supernates to monocyte preparations. The mononuclear cell preparations formed only negligible amounts of PCA in the first 30–60 minutes of incubation, indicating that monocytes were not sufficiently activated to produce PCA in circulating blood. Moreover, monocytes expressed their PCA only in the presence of lymphocytes and needed contact with these cells and some hours of incubation. These observations suggest that monocyte activation and the expression of PCA in vivo are very likely to occur not in the circulating blood but prevalently extravascularly. That monocyte activation and thrombin formation mainly occur in the coronary vessel wall also seems supported by the failure of heparin infusion to fully normalize FPA levels. Several similar observations reported for cancer patients given heparin support the extravascular site of the stimulus for monocytes activation and elevated FPA levels.<sup>59–61</sup> In the coronary atherosclerotic plaque, lymphocytes and monocytes are closely associated with one another in the subendothelial intimal space<sup>31,32,62,63</sup> in addition to the adventitia,<sup>30,32</sup> and in human plaques, tissue factor<sup>64</sup> and tissue factor–producing cells have been identified by in situ hybridization.<sup>65</sup> Moreover, there is evidence that monocyte-macrophages present in the coronary atherosclerotic plaque from patients with

unstable angina but not in those with effort angina are activated because these cells have been found to synthesize tumor necrosis factor,<sup>66–68</sup> whose production is dependent on the presence of proliferating T cells.<sup>69</sup> The present findings do not clarify whether the unknown stimulus that leads to the activation of lymphocytes acts systemically or is restricted to lymphocytes inside the plaque. Similarly, we do not know whether the activated lymphocytes and monocytes from patients with unstable angina are originally inside the vessel wall and then circulate in the blood as a result of plaque rupture or fissuring, although this condition is not necessary because experimental evidence indicates a continuous passage of lymphocytes and monocytes from blood to the vessel wall.<sup>26,70,71</sup>

### Conclusions

Whatever the mechanism(s) and sites of activation, our results support the hypothesis that the outburst of unstable angina represents an acute transient inflammatory state caused by lymphocyte activation intermittently triggered in response to unknown factors. Thus, the primary event in the instability of angina could be the exposure of lymphocytes to an inducer that triggers a series of reactions leading to monocyte activation, increased thrombin generation, and thrombus formation, priming the instability of coronary artery disease.

### References

1. Neri Serneri GG, Gensini GF, Abbate R, Laureano R, Parodi O: Is raised plasma fibrinopeptide A a marker of acute coronary insufficiency? *Lancet* 1980;2:982–983
2. Gallino A, Haeberli A, Bauer HR, Straub PW: Fibrin formation and platelet aggregation in patients with severe coronary artery disease: Relationship with the degree of myocardial ischemia. *Circulation* 1985;72:27–30
3. Theroux P, Latour JG, Leger-Gauthier C, De Lara J: Fibrinopeptide A and platelet factor 4 levels in unstable angina. *Circulation* 1987;75:156–162
4. Bresnahan DR, Davis JL, Holmes DR, Smith HC: Angiographic occurrence and clinical correlates of intraluminal coronary artery thrombus: Role of unstable angina. *J Am Coll Cardiol* 1985;6:285–289
5. Capone G, Wolf NM, Meyer B, Meister SG: Frequency of intracoronary filling defects by angiography in angina pectoris at rest. *Am J Cardiol* 1985;56:403–406
6. Ambrose JA, Winters SL, Arora RR, Eng A, Riccio A, Gorlin R, Fuster V: Angiographic evolution of coronary artery: Morphology in unstable angina. *J Am Coll Cardiol* 1986;7:472–478
7. Ambrose JA, Winters SL, Stern A, Esig A, Teicholz LE, Gorlon R, Fuster V: Angiographic morphology and the pathogenesis of unstable angina pectoris. *J Am Coll Cardiol* 1985;5:609–616
8. Sherman CT, Litvack F, Grundfest W, Lee M, Hickey A, Chaux A, Kass R, Blanche C, Matloff J, Morgestern L, Ganz W, Swan HJC, Forrester J: Coronary angiography in patients with unstable angina. *N Engl J Med* 1986;315:913–919
9. Falk E: Unstable angina with fatal outcome: Dynamic coronary thrombosis leading to infarction and/or sudden death. *Circulation* 1985;71:699–708
10. Maseri A, Chierchia S, L'Abbate A: Pathogenetic mechanisms underlying the clinical events associated with atherosclerotic heart disease. *Circulation* 1980;62(suppl 5):V-3–V-13
11. Fuster V, Badimon L, Cohen M, Ambrose SA, Badimon JJ, Chesebro J: Insights into the pathogenesis of acute ischemic syndromes. *Circulation* 1988;77:1213–1220
12. Chesebro JH, Zoldhelyi P, Fuster V: Pathogenesis of thrombosis in unstable angina. *Am J Cardiol* 1991;68:2B–10B
13. Davies MJ, Thomas AC: Plaque fissuring: The cause of acute myocardial infarction, sudden ischemic death and crescendo angina. *Br Heart J* 1985;53:363–373
14. Davies MJ, Woolf N, Rowles PM, Pepper J: Morphology of the endothelium over atherosclerotic plaque in human coronary arteries. *Br Heart J* 1988;60:459–464

15. Krangel AH, Gertz SD, Roberts WC: Morphologic comparison of frequency and types of acute lesions in the major epicardial-coronary arteries in unstable angina pectoris, sudden coronary death and acute myocardial infarction. *J Am Coll Cardiol* 1991;18:810-818
16. Ambrose JA, Alexandropoulos D: Thrombolysis in unstable angina: Will the beneficial effects of thrombolytic therapy in myocardial infarction apply to patients with unstable angina? *J Am Coll Cardiol* 1989;13:1666-1671
17. Neri Serneri GG, Gensini GF, Poggessi L, Trotta F, Modesti PA, Boddi M, Ieri A, Margheri M, Casolo GC, Bini M, Rostagno C, Carnovali, Abbate R: Effect of heparin, aspirin or alteplase in reduction of myocardial ischaemia in refractory unstable angina. *Lancet* 1990;335:615-618
18. Williams DO, Topol EJ, Califf RM: Intravenous recombinant tissue type plasminogen activator in patients with unstable angina pectoris: Results of a placebo controlled, randomized trial. *Circulation* 1990;82:376-383
19. Bar J, Vermeer F, Verhengt F, Col J, Materne P, Foucault J, De Zwaan C: Thrombolytic therapy has no clinical benefic effect in patients with unstable angina: A placebo controlled study in 159 patients. (abstract) *J Am Coll Cardiol* 1991;17:45A
20. Edwards RL, Rickles FR: Macrophage procoagulants, in Spaet TH (ed): *Progress in Haemostasis and Thrombosis*, vol 7. New York, Grune & Stratton Inc, 1984, pp 183-209
21. Calvin RB, Johnson RA, Mihm MC, Dvorak HF: Role of clotting system in cell mediated immunity: I. Fibrin deposition in delayed skin reactions in man. *J Exp Med* 1973;138:686-698
22. Edwards RL, Rickles FR: Delayed hypersensitivity in man: Effects of systemic anticoagulation. *Science* 1978;200:541-543
23. Hardin JA, Cronlund M, Haber E, Block J: Activation of blood clotting in patients with systemic lupus erythematosus: Relationship to disease activity. *Am J Med* 1978;65:430-436
24. Brochier ML, Raynand P, Rioux P, Charbonnier B, Desveaux B, Pacoret G: Thrombosis and thrombolysis in unstable angina. *Am J Cardiol* 1991;68:105-109
25. Edwards RL, Levin JB, Green R, Duffy M, Matthews E, Brande W, Rickles FR: Activation of blood coagulation in Crohn's Disease. *Gastroenterology* 1987;92:329-337
26. Schwartz CJ, Mitchell JRA: Cellular infiltration of the human arterial adventitia associated with atheromatous plaque. *Circulation* 1962;26:73-78
27. Vlodaver Z, Edwards JE: Pathology of coronary atherosclerosis. *Prog Cardiovasc Dis* 1971;14:256-274
28. Ross R, Wight TN, Strandness E, Thiele B: Human atherosclerosis: I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. *Am J Pathol* 1984;114:79-93
29. Gown AM, Tsukada T, Ross R: Human atherosclerosis. Immunohistochemical analysis of cellular composition of human atherosclerotic lesions. *Am J Pathol* 1986;125:191-207
30. Kochi K, Takebayashi S, Hikori T, Nobuyoshi M: Significance of adventitial inflammation of the coronary artery in patients with unstable angina: Results at autopsy. *Circulation* 1985;71:709-716
31. Sato T: Increased subendothelial infiltration of the coronary arteries with monocytes/macrophages in patients with unstable angina. *Atherosclerosis* 1987;68:191-197
32. Baroldi G, Silver MD, Mariani F, Giuliano G: Correlation of morphological variables in the coronary atherosclerotic plaque with clinical patterns of ischemic heart disease. *Am J Cardiovasc Pathol* 1988;2:159-172
33. Rickles FR, Levin JA, Hardin JA, Barr CF, Conrad ME: Tissue factor generation by human mononuclear cells: Effects of endotoxin and dissociation of tissue factor generation from mitogenic response. *J Lab Clin Med* 1977;89:792-803
34. Rothberger H, Zimmermann TS, Spiegelberg HL, Vanhan JE: Leukocyte procoagulant activity: Enhancement of production in vitro by IgG and antigen antibody complexes. *J Clin Invest* 1977;59:459-466
35. Muhlfelder TW, Niemitiz J, Krentzer D, Beebe D, Word P, Rosenfield SI: C5 chemotactic fragment leukocyte production of tissue factor activity. *J Clin Invest* 1979;63:147-150
36. Edwards RL, Rickles FR, Cronlund M: Abnormalities of blood coagulation in patients with cancer: Mononuclear cell tissue factor generation. *J Lab Clin Med* 1981;98:917-928
37. Dean RT, Prydz H: Inflammatory particles stimulate thromboplastin production by human monocytes. *Thromb Res* 1983;30:357-367
38. Lorenzet R, Peri G, Locati D, Allavena P, Colucci M, Semeraro N, Mantovani A, Donati MB: Generation of procoagulant activity by mononuclear phagocytes: A possible mechanism contributing to blood clotting activation within malignant tissue. *Blood* 1983;62:271-273
39. Schwartz BS: Antigen-induced monocyte procoagulant activity: Requirement for antigen presentation and histocompatibility leukocyte antigen-DR molecules. *J Clin Invest* 1985;76:970-977
40. Gregory SA, Kornbluth RS, Helin H, Remold HG, Edgington TS: Monocyte procoagulant inducing factor: A lymphokine involved in the T cell-instructed monocyte procoagulant response to antigen. *J Immunol* 1986;137:3231-3239
41. Braunwald E: Unstable angina: A classification. *Circulation* 1989;80:410-414
42. Theroux P, Ouimet H, McCans J, Latour JG, Joly P, Levy G, Pelletier E, Juneau M, Stasiak J, De Guise P, Pelletier GB, Rinzler D, Waters D: Aspirin, heparin or both to treat acute unstable angina? *N Engl J Med* 1988;319:1105-1111
43. Boyum A: Isolation of lymphocytes, granulocytes and macrophages. *Scand J Immunol* 1976;5(suppl 5):9-15
44. Yam LT, Li CY, Crosby WH: Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 1971;55:283-290
45. Bevilacqua MP, Amrani D, Mosesson MW, Bianco C: Receptors for cold-insoluble globulin (plasma fibronectin) on human monocytes. *J Exp Med* 1981;153:42-60
46. Helin HJ, Fox RI, Edgington TS: The instructor cell for the human procoagulant monocyte response to bacterial lipopolysaccharide is a Leu-3a+ T cell by fluorescence-activated cell sorting. *J Immunol* 1981;131:749-752
47. Helin H, Edgington TS: Allogenic induction of the human T cell-instructed monocyte procoagulant response is rapid and is elicited by HLA-DR. *J Exp Med* 1983;158:962-975
48. Otnaess AB, Prydz H, Bjorklid E, Berre A: Phospholipase C from *Bacillus cereus* and its use in studies of tissue thromboplastin. *Eur J Biochem* 1972;27:238-243
49. Zacharski LR, Rosenstein R, Phillips G: Concanavalin A inhibition of tissue factor (thromboplastin) activity. *Blood* 1974;6:783-787
50. Gordon SG, Cross BA: A factor X-activating-cysteine protease from malignant tissue. *J Clin Invest* 1981;67:1665-1671
51. Gaffney PJ, Mahmoud JF, Fossari CA, Spitz M: A novel radioimmuno-metric approach to the assay of components of human haemostasis: I. assay of plasma fibrinopeptide A levels. *Thromb Res* 1980;19:815-822
52. Soria J, Soria C, Ryckewaert JJ: A solid phase immunoenzymological assay for the measurement of human fibrinopeptide A. *Thromb Res* 1980;20:425-435
53. American Heart Association: Committee Report: A reporting system on patients evaluated for coronary artery disease. *Circulation* 1975;51:5-7
54. Berk BC, Weintraub WS, Alexander RW: Elevation of C-reactive protein in active coronary disease. *Am J Cardiol* 1990;65:168-172
55. Rab ST, Alexander RW, Ansari AA: Evidence for activated circulating macrophage/monocytes in unstable angina. (abstract) *J Am Coll Cardiol* 1990;15:168A
56. Levy GA, Edgington TS: Lymphocyte cooperation is required for amplification of macrophage procoagulant activity. *J Exp Med* 1980;151:1232-1244
57. Levy GA, Schwartz BS, Edgington TS: The kinetics and metabolic requirements for direct lymphocyte induction of human procoagulant monokines by bacterial lipopolysaccharides. *J Immunol* 1981;127:357-363
58. Schwartz BS, Edgington TS: Immune complex-induced human monocyte procoagulant activity: I. A rapid unidirectional lymphocyte-instructed pathway. *J Exp Med* 1981;154:892-906
59. Rickles FR, Edwards RL, Barb C, Cronlund M: Abnormalities of blood coagulation in patients with cancer: Fibrinopeptide A and tumor growth. *Cancer* 1981;51:301-307
60. Yudelman I, Greenberg J: Factors affecting fibrinopeptide A levels in patients with venous thromboembolism during anticoagulant therapy. *Blood* 1982;59:787-792
61. Edwards RL, Klaus M, Matthews E, McCullen C, Bona RD, Rickles FR: Heparin abolishes the chemotherapy-induced increase in plasma fibrinopeptide A levels. *Am J Med* 1990;89:25-28
62. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK: Regional accumulation of T cells, macrophages and smooth muscle cells in the human atherosclerotic plaque. *Atherosclerosis* 1986;6:131-138
63. Emeson EE, Robertson AL: T lymphocytes in aortic and coronary intimas: Their potential role in atherogenesis. *Am J Pathol* 1988;130:369-376
64. Drake TA, Morrissey JH, Edgington TS: Immunohistochemical detection of tissue factor in human atherosclerotic plaques. (abstract) *Circulation* 1989;80(suppl II):II-182



65. Wilcox JN, Smith KM, Schwartz SM, Gordon D: Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A* 1989;86:2839-2843
66. Arbustini E, Grasso M, Diegoli M, Pucci A, Bramerio M, Ardisino D, Angoli L, De Servi S, Bramucci E, Munini A, Minzioni G, Viganó M, Specchia G: Coronary atherosclerotic plaques with and without thrombus in ischemic heart syndromes: A morphologic, immunohistochemical and biochemical study. *Am J Cardiol* 1991; 68:36B-50B
67. Van der Wal AC, Das PK, Van de Berg DB, Van der Loos CM, Becker AE: Atherosclerotic lesions in humans: In situ immunophenotypic analysis suggesting an immune mediated response. *Lab Invest* 1989;61:166-170
68. Barath P, Fishbein MC, Cao J, Berenson J, Helfant R, Forrester S: Detection and localization of tumor necrosis factor in human atheroma. *Am J Cardiol* 1990;65:297-302
69. Debets JMH, Van der Linden CS, Spronken IEM, Buurman WA: T cell-mediated production of tumor necrosis factor-alpha by monocytes. *Scand J Immunol* 1988;27:601-608
70. Joris I, Zand T, Nunnari JJ, Krolikowski FJ, Majno G: Studies on the pathogenesis of atherosclerosis: I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am J Pathol* 1983;113:341-358
71. Harlan JH: Leucocyte-endothelial interactions. *Blood* 1985;65: 513-525