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Leukocyte activation in patients with venous insufficiency

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Purpose: **Cell activation may play an important role in the production of venous insufficiency, just as leukocytes participate in the cause of venous ulcer. If activated, monocytes observed on venous endothelium can migrate into the venous wall and produce toxic metabolites and free oxygen radicals that may participate in valve destruction and venous wall weakening. At present, it remains uncertain to what degree leukocytes are actually activated in patients. This study was designed to explore the level of activation and to examine whether patient plasma contains an activator that leads to leukocyte activation of unstimulated naive leukocytes from volunteers without venous insufficiency disease.**

Methods: **Twenty-one patients (4 men, 17 women), who ranged in age from 34 to 69 years (mean age, 53.2 years), with chronic venous disease were compared with 16 healthy control volunteers (4 men, 12 women), who ranged in age from 18 to 65 years (mean age, 48.4 years). All the patients underwent evaluation with Doppler ultrasound scanning and were classified with the CEAP score.[1] Nearly all the patients who smoked or were hypertensive were excluded. The blood types (ABO and Rh) of the controls were matched to the study group. Isolates of patient whole blood, plasma, or leukocytes were incubated with isolates of control whole blood, plasma, or leukocytes to separate actual activation from spontaneously observed activation. The granulocyte activation was measured with nitroblue tetrazolium (NBT) reduction and quantitation of granulocyte pseudopod formation. Hydrogen peroxide production in patient plasma was measured with a recently developed electrode method.**

Results: **Leukocytes from healthy blood and patient plasma had significantly higher NBT-positive granulocyte counts than either patient blood, healthy blood, or patient blood incubated in healthy plasma. In a comparison of patient groups across the CEAP classes, the NBT-positive granulocyte counts were significantly greater in classes 4, 5, and 6 than in classes 2 and 3 ($P < .001$). Pseudopod formation was significantly greater in mixtures of granulocytes in healthy blood and patient plasma than in all other groups. There was no difference in the level of pseudopod formation in control leukocytes incubated with patient plasma in patients across the CEAP spectrum. The patient plasma produced significantly higher hydrogen peroxide values than did the controls.**

Conclusion: **These results suggest that patient plasma may contain an activating factor for granulocytes. The finding that activated neutrophils were fewer in number in patient whole blood than in healthy blood incubated in patient plasma could suggest that activated neutrophils in patients with chronic venous insufficiency might be trapped in the peripheral circulation. It is unknown what factors in the plasma might induce activation of naive neutrophils, but such activators could possibly be important in the pathogenesis of primary venous dysfunction and the development of chronic venous insufficiency. (J Vasc Surg 1999;30:148-56.)**

Direct inspection of saphenous veins from patients with venous dysfunction has shown that their valves are fewer in number[2-4] and that the existing valves show evidence of damage.[5] This damage is seen as shortening, thickening, perforation, or splitting of the leaflets.[6,7] We have shown that, in saphenous veins removed during surgical correction of venous insufficiency, monocytes are found on the endothelium and in vein wall interstitium intimately associated with interstitial macrophages and with intracellular adhesion molecules (ICAM-1).[8] This evidence suggests that activated leukocytes may be related to valvular damage and perhaps to weakness of the vein wall.

Because activated cells may release proteolytic enzymes or reactive oxygen intermediates that cause

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such tissue damage, the hypothesis was proposed that soluble markers of leukocyte adhesion and activation could be detected in the plasma of individuals with venous insufficiency. If such an activator were present in the plasma of patients, a potential driving mechanism for venous insufficiency might be identified that could become a target for future interventions. Therefore, this study was carried out to obtain direct evidence for cell activation in venous blood samples of patients with venous insufficiency.

Table I. Demographic data of study patients

No. of individuals	21
Men:women	4:17
Mean age (years)	53.2 (range, 34 to 69)
CEAP class 3	14
CEAP class 4	6
CEAP class 5	1
Current smoker	3
Obesity	1
Hypertension	0
Birth control pill	2
Premarin	5

PATIENTS AND METHODS

All the procedures for this study were conducted with approval of the Human Subjects Committee of the University of California, San Diego, and the Scripps Memorial Hospital, La Jolla. A total of 21 patients (4 men, 17 women), who ranged in age from 34 to 69 years (mean age, 53.2 ± 9.8 years), with chronic venous disease who underwent surgical treatment were studied (Table I). All the patients were classified with the CEAP classification, and all underwent evaluation for reflux of the greater saphenous vein and elsewhere with Doppler ultrasound scanning (Table I). For the most part, the subjects who smoked cigarettes or were hypertensive were excluded because of their known nonspecific activation of leukocytes.[9] The patients with known or suspected inflammatory disorders also were excluded. Sixteen healthy control volunteers (4 men, 12 women), who ranged in age from 18 to 65 years (mean, 48.4 ± 14.2 years), donated blood for the studies. The volunteers were interviewed to exclude inflammatory disease and were screened for venous disease. After informed consent was given and after the subject was in the supine position for 20 minutes, 20 mL of blood was drawn into heparinized tubes from the antecubital vein of the volunteers. This precaution was taken to prevent the stimulating effects of the standing position on leukocyte activation.[10] Also, the patients for surgery had a 20-minute interval of lying on the operating table before the blood samples were drawn. ABO and Rh testing was performed on all the samples so that ABO and Rh blood type could be matched between patients and controls. All the samples were stored on ice and handled gently to minimize spontaneous activation in vitro.

Blood sample preparation

The healthy granulocytes in control whole blood (NG) were tested. Likewise, the patient granulocytes in whole blood were tested (PG). These cells were tested directly without separation to assess the level of activation in the circulation. The remaining blood sample was centrifuged at 3000 rpm at 4°C for 30 minutes to prepare platelet-poor plasma, which then was centrifuged again in the same fashion. The resulting control plasma (Cp) or patient plasma (Pp) was mixed with control whole blood containing naive granulocytes (NG) in a volume ratio of 4:1. The purpose of this exchange of plasma was to expose naive granulocytes (NG) from a volunteer without evidence of venous disease or significant leukocyte activation to the plasma of patients or the plasma of controls. From this point, we will refer to these leukocytes as naive leukocytes to emphasize that these cells were from asymptomatic individuals. The volume ratio of 4:1 was selected to ensure that the naive leukocytes were suspended in almost 90% patient plasma, assuming a hematocrit of 40%. The mixture was incubated at 37°C for 15 minutes before measurement of neutrophil activation.

As a negative control, a healthy plasma sample from an AB+ healthy control was mixed with plasma from controls in the same volume ratio of 4:1. Control plasma prepared for comparison with patient plasma was immediately frozen in liquid nitrogen, stored at –70°C, and tested as described previously. The mixtures of negative control and positive test mixtures were incubated and studied in parallel.

Finally, we examined nitroblue tetrazolium (NBT) reduction and pseudopod formation in four different groups: (1) control whole blood with healthy granulocytes (NG) alone, (2) naive granulocytes mixed with control plasma (NG + Cp), (3) patient whole blood with patient granulocytes (PG) alone, and (4) naive granulocytes mixed with patient plasma (NG + Pp).

Experimental procedure

NBT reduction. NBT solution (0.1 mL; 1 mg NBT in 1 mL distilled water; Sigma, St Louis, Mo) was incubated with 0.1 mL of fresh sample at 37°C for 10 minutes and then stored for 10 minutes at room

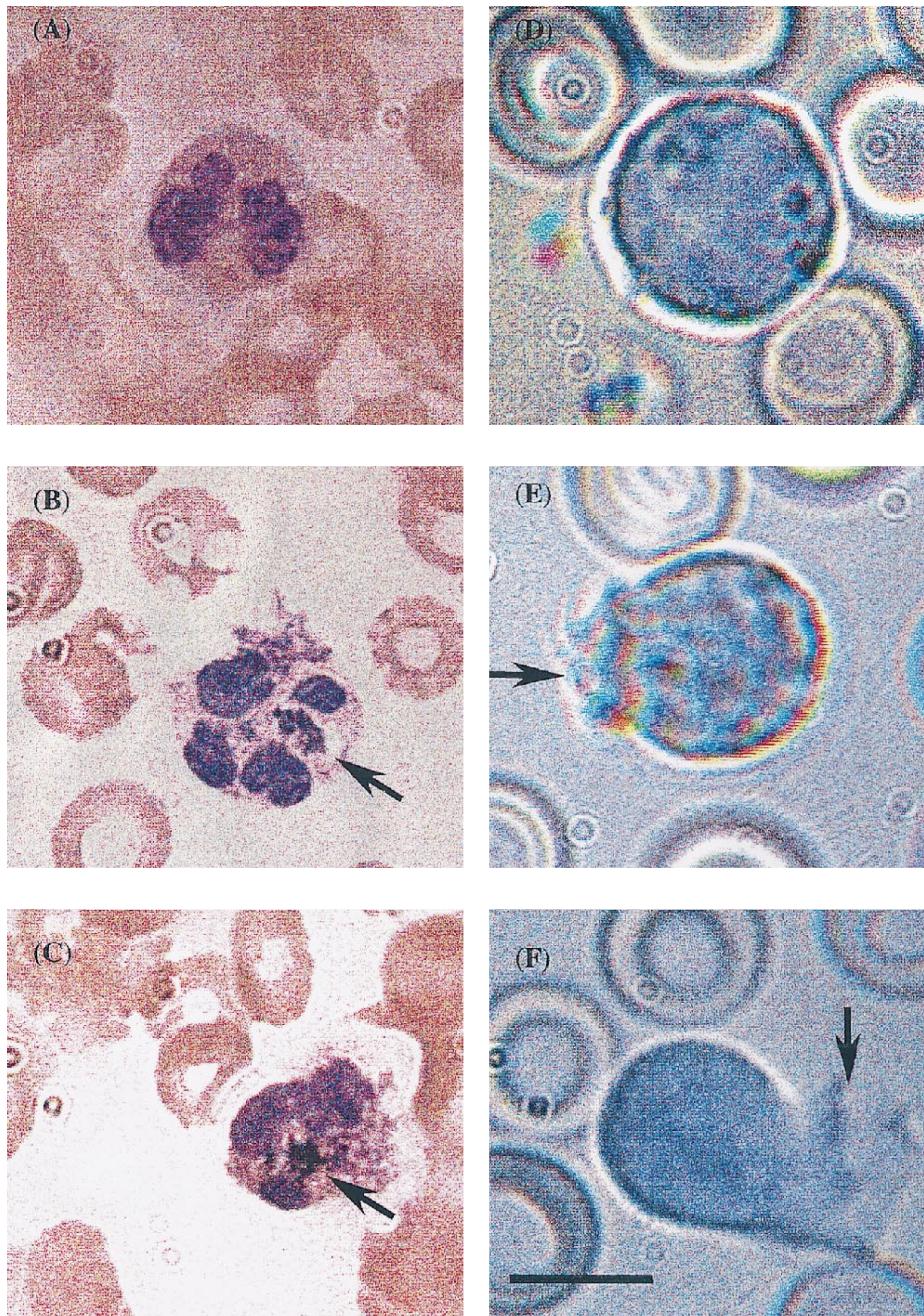

Fig 1. Micrographs of polymorphonuclear granulocytes showing tetrazolium crystal formation after nitroblue tetrazolium reduction **(A,B,C)** and projection of pseudopods **(D,E,F)**. Control blood of individuals without symptoms **(A,D)**, patient whole blood **(B, E)**, and naive granulocytes incubated in patient plasma **(C, F)**. *Bar* represents 10 μm. Magnification is same for all panels.

temperature (23°C to 24°C). A blood smear was prepared and dried. The slides were treated with 1 mL of Wright's stain (0.3 w/v buffered at pH 6.9 in methanol; Sigma) for 15 seconds and 1 mL of distilled water for 30 seconds at room temperature, rinsed with distilled water, and then dried. Granulocytes that contained the characteristic black crystals of formazan from which NBT was reduced by superoxide generation in their cytoplasm were considered NBT positive. However, granulocytes that contained platelets and

had formazan were not counted as positive (Fig 1*A to C*). A total of 100 neutrophils per sample were counted three times. The count is reproducible on average within 1% if repeated by the same investigator. NBT reduction can be blocked with superoxide dismutase.[11]

Pseudopod formation. After the removal of an aliquot of blood for the NBT test, the samples were incubated for another 15 minutes at 37°C and separated into plasma and cells with sedimentation at 1 g. Then, 0.1 mL of the supernatant layer containing plasma, leukocytes, platelets, and sporadic erythrocytes was removed and added to 0.1 mL of 1% glutaraldehyde (Fisher Scientific, Fair Lawn, NJ) in 0.1 mol/L cacodylate buffer ($Na[CH_3]2AsO_2/3H_2O$ in distilled water; Ted Pella Inc, Redding, Calif). The cells were fixed for 30 minutes, and then crystal violet in 70 mmol/L phosphate buffer was added to stain the leukocyte nuclei in wet mount preparations. Freely suspended leukocytes with pseudopodia (Fig 1*D to F*) were identified with their segmented nuclei and presence of cytoplasmic granules. A total of 100 cells were counted three times repeatedly. Cells with pseudopod projection greater than 1 μm were considered positive. The count is reproducible on average within 2% if repeated by the same investigator.

Hydrogen peroxide production in plasma. Levels of hydrogen peroxide production in plasma were measured with an electrode as described previously.[12] Of the 21 patients in the study, five donated blood for the H_2O_2 examination. Eleven other patients also donated blood samples. Three men and 13 women (mean age, 46 ± 9 years) and 14 controls (10 men, 4 women; mean age, 45 ± 4 years) without evidence of venous disorders or hypertension were enrolled (Table II). Briefly, fresh samples were prepared for measurement with the placement of 1.5 mL of blood in a 2-mL centrifuge tube followed by incubation at 37°C for 10 minutes and centrifugation at 500 g for 10 minutes. After the sample preparation, either sodium azide (20 μL of a 2 mol/L) or catalase (20 μL containing 0.25 mg of the enzyme) was added to approximately 0.75 mL of the plasma layer. The plasma then was swiftly stirred without mixing the buffy coat and red blood cell layer with the plasma. The electrode was placed in the plasma, and its output was recorded for 10 minutes. Measurements after the addition of sodium azide and catalase were each carried out in duplicate. The current obtained from the plasma sample with catalase was subtracted from the current with sodium azide. The difference between these currents was ascribed to hydrogen peroxide and directly calibrated with additions of known quantities of hydrogen peroxide to buffer or human plasma with sodium azide.[12]

Table II. Demographic data of H_2O_2 study patients

No. of individuals	16
Men:Women	3:13
Mean age (years)	45 (range, 31 to 59)
CEAP class 3	14
CEAP class 4	1
CEAP class 5	0
CEAP class 6	1
Current smoker	1
Diabetes	0
Obesity	4
Hypertension	0
Birth control pill	2
Premarin	3

Statistics. All the measurements are presented as mean ± standard deviation. Mann-Whitney test was performed for comparisons between control blood and patient blood or mixtures of naive granulocytes with control or patient plasma. Wilcoxon paired signed rank test was performed for comparisons between naive leukocytes after incubation in patient and control plasma and for pair comparisons of patient (or control) whole blood with naive leukocytes incubated in patient (or control) plasma. Pearson product moment correlations were used to test relationships between NBT-positive values and pseudopod formation values. In addition, simple regression was performed to evaluate between NBT reduction and pseudopod formation values. Statistically significant differences were determined at the P level of .05. All analyses were performed with commercial software (StatView Version 4.5 for MacIntosh, Abacus Concepts Inc, Berkeley, Calif).

RESULTS

NBT reduction. The NBT-positive cells were counted in each sample. There was no difference in NBT reduction between the control whole blood or the naive granulocytes mixed with control plasma (5.40 ± 1.91 vs 8.69 ± 2.84). This provided a negative control. Also, patient whole blood alone (7.86 ± 6.90) compared with control whole blood or naive cells mixed with control plasma exhibited no differences in NBT reduction. This provided negative controls related to the manipulation of the samples.

Increased NBT reduction as a result of superoxide production was detectable when patient plasma was applied to naive granulocytes (Fig 2). Control blood cells containing naive leukocytes mixed with patient plasma (14.3 ± 6.29) showed a significantly greater level of NBT reduction than all of the other

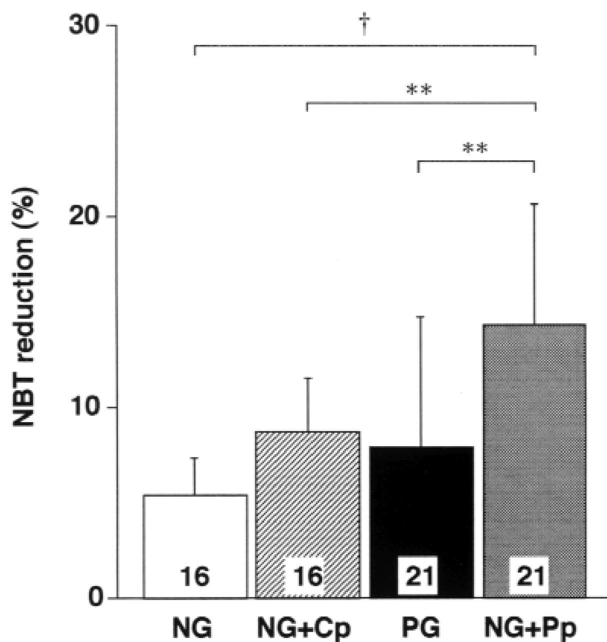

Fig 2. Levels of nitroblue tetrazolium *(NBT)* reduction. *NG,* Naive granulocytes; *Cp,* control plasma; *PG,* patient granulocytes; *Pp,* patient plasma.
$^{**}P < .001$ and †$P < .0001$, compared with naive granulocytes in patient plasma.

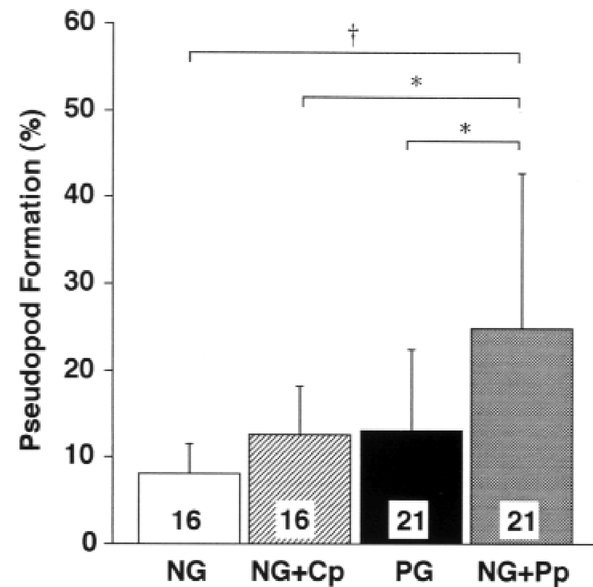

Fig 3. Levels of granulocyte pseudopod formation. *NG,* Naive granulocytes; *Cp,* control plasma; *PG,* patient granulocytes; *Pp,* patient plasma.
$^{*}P < .05$ and †$P < .0001$, compared with naive granulocytes in patient plasma.

combinations. The plasma from patients with chronic venous insufficiency, CEAP classes 4 to 6, stimulated about 2.7 times greater NBT-positive granulocyte counts than did control plasma.

By means of microscopy, formazan crystals in leukocytes were seen to be spotty and scattered in all groups when leukocytes were activated. In contrast, when naive cells were mixed with patient plasma, the crystals were frequently clustered in larger aggregates (Fig 1*C*).

Pseudopod formation. There was no statistically significant difference in frequency of granulocytes with pseudopod formation in control whole blood as compared with naive blood cells mixed with control plasma (8.1 ± 3.4 vs 12.6 ± 5.5; $P > .05$). Also, patient whole blood compared with control whole blood was not statistically different (13.0 ± 9.45; $P > .05$).

In contrast, a significant increase in pseudopod formation in the naive granulocytes was observed after incubation with patient plasma ($P < .001$). Control blood cells mixed with patient plasma showed an average of 25.0 ± 14.7 leukocytes, with pseudopod formation per 100 cells. This mixture also had a significantly higher level of pseudopod formation than did the mixture of naive cells and control plasma (Fig 3). The plasma from patients caused about two times greater levels of pseudopod formation in naive granulocytes than were observed in granulocytes of control blood without stimulation.

When examined with photomicrographs, pseudopod projections in leukocytes from control whole blood, naive cells mixed with control plasma, and patient whole blood were small and narrow. In contrast, the pseudopod projections in naive cells mixed with patient plasma were comparatively large and wide and pointed in multiple directions (Fig 1*F*).

Level of activation in CEAP clinical classes. There were no differences in NBT reduction and pseudopod formation in untreated blood from patients in class 2 as compared with class 3 or among classes 4, 5, and 6. In contrast, there were significant differences in NBT reduction and pseudopod formation between granulocytes from patient whole blood and naive granulocytes mixed with patient plasma among classes 2 and 3 and classes 4, 5, and 6 (Figs 4, 5). Patient plasma from classes 4, 5, and 6 caused comparatively greater granulocyte activation in terms of superoxide production and actin polymerization than did patient plasma from classes 2 and 3. However, the granulocytes in patient whole

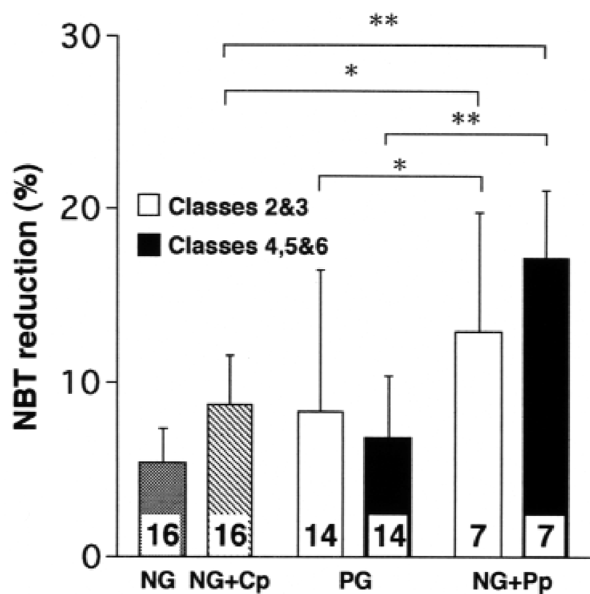

Fig 4. Comparison of nitroblue tetrazolium *(NBT)* reduction. Patient samples were grouped into CEAP classes 2 and 3 and classes 4, 5, and 6.
NG, Naive granulocytes; *Cp,* control plasma; *PG,* patient granulocytes; *Pp,* patient plasma.
**P* < .05.
***P* < .001.

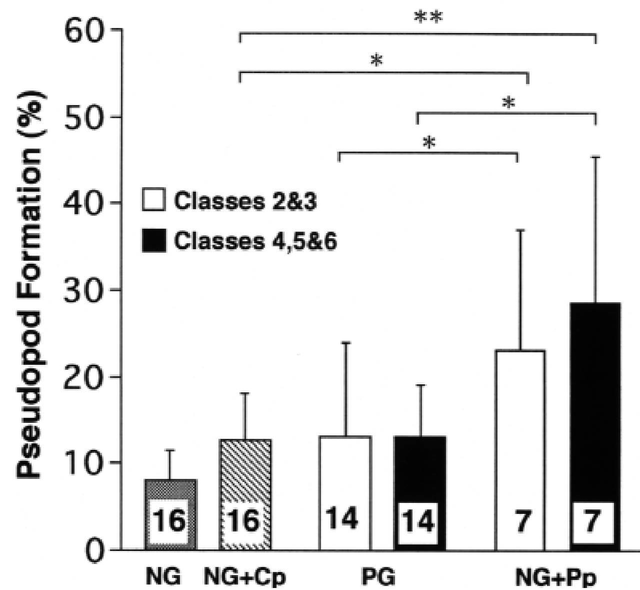

Fig 5. Comparison of granulocyte pseudopod formation. Patient samples were grouped into CEAP classes 2 and 3 and classes 4, 5, and 6.
NG, Naive granulocytes; *Cp,* control plasma; *PG,* patient granulocytes; *Pp,* patient plasma.
**P* < .05.
***P* < .001.

blood from the various clinical classes showed no differences from one another with regard to NBT reduction and pseudopod formation.

When naive neutrophils were mixed with plasma from controls, the level of neutrophil activation measured with NBT reduction and pseudopod formation was significantly less than when the naive neutrophils were mixed with patient plasma from either classes 2 and 3 or classes 4, 5, and 6 ($P < .05$).

Correlation between NBT reduction and pseudopod formation in chronic venous insufficiency. There was no correlation between NBT reduction and pseudopod formation in untreated control whole blood (r = 0.24, $P = .93$) or in control blood leukocytes mixed with control plasma (r = 0.28, $P = .29$). Nor was there any correlation between NBT reduction and pseudopod formation in patient whole blood from classes 4, 5, and 6 (r = 0.33, $P = .50$). There was no correlation between the mixture of control blood cells and patient plasma from classes 2 and 3 (r = 0.34, $P = .24$) and from classes 4, 5, and 6 (r = 0.26, $P = .59$). There was a weak correlation between the two forms of activation in whole blood from patients in classes 2 and 3 (r = 0.535, $P = 0.47$).

Plasma hydrogen peroxide production. The average hydrogen peroxide production in the plasma of patients was about 50% greater than in controls (Fig 6). There was no significant correlation between the number of NBT-positive neutrophils and the hydrogen peroxide production in plasma.

DISCUSSION

The results of the current study suggest that some aspects of leukocyte function are upregulated in the circulation of patients with venous insufficiency. The plasma exchange studies in which patient plasma is applied to naive granulocytes derived from individuals without any evidence of cardiovascular dysfunction or venous disease suggest that the patients carry a factor in the plasma that is related to the upregulation. Granulocytes from patients exhibit reduced levels of activation after resuspension in plasma from controls. The plasma of patients serves as a source for free radical production.

We noted a lack of correlation between NBT reduction and pseudopod formation. This was also seen when CEAP classes 2 and 3 were compared with CEAP classes 4, 5, and 6. This is in agreement with similar observations made by us in studies with exper-

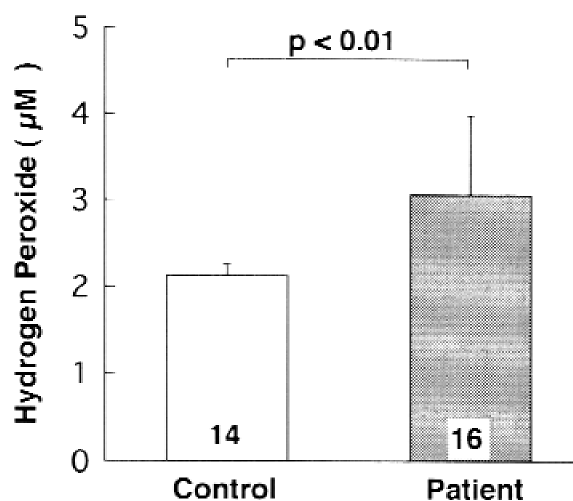

Fig 6. Hydrogen peroxide production levels in patients with chronic venous insufficiency and in asymptomatic controls.

imental animals in ischemic conditions. Unpublished observations are in contrast to the strong correlation between different forms of activation after stimulation of leukocytes in vitro. After in vitro stimulation, different forms of activation follow a similar time course. Direct observation of leukocytes in the microcirculation suggests that cells are in different stages of activity in line with the lack of correlation in the present study.

The activation of naive cells appears to induce oxygen-free radical production as indicated by the NBT reaction. Direct evidence of hydrogen ion production obtained with the electrochemical hydrogen peroxide measurements confirms this hypothesis. Further evidence of activation of naive granulocytes was provided with the observation of increased pseudopod formation in these cells when they were exposed to patient plasma. Although the former reaction of hydrogen ion production parallels the severity of venous disease, the latter reaction of pseudopod formation does not.

The current selection of pseudopod formation and NBT reduction as measures of cell activation is motivated by two considerations. First, to detect the levels of cell activation as they exist in vivo, in vitro cell isolation techniques must be minimized. Also, it is essential that fresh blood samples be tested. Both the NBT reduction test and the rapid fixing of cells for detection of spontaneous pseudopod formation serve such a purpose. Second, both forms of activation, superoxide formation for NBT reduction and actin polymerization during pseudopod projection, indicate important pathophysiologic processes in the circulation. Cells that exhibit pseudopod formation have a higher propensity to be trapped in the microcirculation,[13-15] and superoxide formation constitutes one of the first steps in the oxidative stress cascade.[16] As such, it is an important element in the undesirable aspect of leukocyte activation, it is encountered in a variety of ischemic diseases,[17,18] and it may constitute a significant risk factor for the development of venous valve damage and subsequent insufficiency. Direct evidence of superoxide anion production obtained with the electrochemical hydrogen peroxide measurements serves as additional support of this hypothesis.

Activated leukocytes may be more likely to adhere to the endothelium in the pockets of venous valves and may exhibit more enhanced levels of cytotoxity. This hypothesis is in line with the increased infiltration of leukocytes observed in the venous wall and valve leaflet of patients with venous dysfunction.[8] Also, this may be associated with the enhanced expression of endothelial adhesion molecules.[19] Activation may be stimulated by the production of specific humoral factors, such as platelet-activating factor or complement fragments,[20,21] by depletion of endogenous deactivating factors,[17,22] or by a mere alteration in the fluid shear stress. Shear changes are sufficient to affect gene expression in endothelial cells or leukocytes.[23] Leukocytes on the endothelium and in the venous wall have been found to be more numerous on the proximal valve surface and adjacent wall where venous hypertension is the greatest.

Examination of healthy veins and varicose veins with light and transmission electron microscopy has been reported by Obitsu et al.[24] They concluded that valve failure started with depression of the commissure of the valve with subsequent expansion of the space between the valve cusps. In their study, elongation of the cusps with bulbous thickening of the free-edge cusp was noted and hyperplasia of the collagen fibers in the valve cusp was detected. Our own studies have shown a deterioration of collagen in the valves and shortening of the valves, suggesting valve damage.[8]

Coleridge Smith[25] has presented a detailed immunohistochemical study of eight varicose saphenous vein samples and four healthy controls by Wilkinson et al.[26] Few infiltrating cells were found, but those that were present were tissue macrophages carrying the FCR1 receptor. Our own studies have revealed a greater influx of cells, but they have been monocytes that become macrophages after penetration of the endothelium.[8]

Wilkinson et al[26] apparently also found class II major histocompatibility antigens on the endothelium and did find macrophages in smooth muscle cells in the thickened tunica media. They found upregulation of HLA-D/DR in all three cell types, but there was a preferential upregulation of HLA-D/DQ in the smooth muscle cells. In general, the function of the class II locus is to present processed antigens to T-cells. The role of the D/DQ at this site is uncertain and may be involved in the maintenance of a chronic inflammatory process. The site of D/DQ expression within the thickened media suggests a possible active role within the development of the venous insufficiency disorder.

Judging from the levels of activation alone, it may be reasonable to combine limbs with CEAP classes 4, 5, and 6 as a single clinical entity because they all have manifestations of inflammation and even infection. Classes 4 and 5 exhibit inflammation, and class 6 implies infection as well. Classes 4, 5, and 6 represent a group of individuals whose plasma, enhanced possibly by the presence of inflammation, is capable of activating naive donor leukocytes. But even patient plasma from more modest forms of venous insufficiency, CEAP classes 2 and 3, show signs of cell activation. This observation suggests that even in an earlier stage of development of venous dysfunction, several markers for inflammation are present. A fundamental issue in venous insufficiency is identification of the mechanism for this early form of activation. The activation process may constitute a potential new target for pharmacologic or other intervention. A similar activation of circulating leukocytes has also been observed in claudication.[27]

It is possible that, during the exposure of naive granulocytes to patient plasma, a stabilizing effect or an inhibitory factor on the cells is removed rather than an activator added. With this hypothesis, patient granulocytes do not respond because they are unable to do so. Further, the cell activation in this study might be related to venous insufficiency or to subclinical inflammation alone or to a combination of both.

Clinically, leukocytes that project pseudopods have a reduced probability of circulating systemically. This leads to a situation in which the full extent of cell activation may not necessarily be identified from tests performed on autologous systemic venous blood samples. Our results indicating higher levels of activation after incubation of naive granulocytes with patient plasma than detected in autologous blood are in line with the hypothesis that the fully activated cells do not circulate and are trapped in the peripheral circulation. If fully activated cells are trapped, one cannot assume that the pool of circulating leukocytes is a well-mixed pool and exhibits a uniform degree of activation.

It should be noted that in the analysis of results, some of the observed values are small and the standard deviations are large. This may be a result of the small number of observations made on biologically diverse individuals. Also, it could be hypothesized that venous hypertension is associated with the phenomenon of leukocyte trapping. This would explain the absence of such trapping in the upper extremities and portal system in patients with severe venous insufficiency. In addition, this may explain the findings of severe chronic venous insufficiency in the upper extremities that contain a functioning arteriovenous fistula and proximal subclavian venous obstruction.[28,29]

We expected that the level of activation produced by patient plasma on naive leukocytes would correlate with the severity of venous insufficiency. The CEAP classification stratifies limbs according to the severity of their clinical findings, but patient plasma samples from those individuals with the most florid three classes of venous insufficiency were indistinguishable from one another. This may be a sign that chronic upregulation may serve as a cell injury mechanism. Restructuring of venous vessels may depend on the duration of an upregulated state rather than only the severity of the upregulation in the venous blood. This is suggested by the fact that clinical manifestations of venous insufficiency do progress with time (eg, varicose veins and telangiectasias enlarge). A shift from one CEAP class to the next does not necessarily necessitate higher levels of activation but only a longer duration of action. Patient plasma, even in the lower CEAP classification with uncomplicated varicose veins, did differ from that of healthy individuals.

The evidence that the plasma of patients contains an activator for granulocytes constitutes a particular challenge. Although the specific biochemical identity of this factor is unknown, it is possible that this factor may not serve to upregulate only granulocytes but also other circulating cells and endothelial cells and possibly even interstitial cells. The factor may be important in the pathogenesis of primary venous dysfunction and the cause of chronic venous insufficiency.

CONCLUSIONS

These results suggest that patient plasma may contain an activating factor for granulocytes. The finding that activated neutrophils were fewer in number in patient whole blood than in healthy

blood incubated in patient plasma could suggest that activated neutrophils in patients with chronic venous insufficiency might be trapped in the peripheral circulation. It is unknown what factors in the plasma might induce activation of naive neutrophils, but such activators could possibly be important in the pathogenesis of primary venous dysfunction and the development of chronic venous insufficiency.

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