A Retrospective Study of Radiolabeled Granulocyte Kinetics in Patients with Systemic Vasculitis

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Patients with systemic vasculitis, including Wegener's granulomatosis (WG) and microscopic polyarteritis (MP), may undergo white cell scanning for the investigation of infective complications and/or occult fever. In a retrospective study of 12 patients with systemic vasculitis (six each of WG and MP), all with renal disease, we observed increased diffuse lung radioactivity soon after the injection of ^{111}In-labeled granulocytes or ^{99m}Tc-HMPAO-labeled leukocytes in all patients with WG and in three with MP. Lung activity was quantified by comparison with the liver or spleen. The lung:liver count rate ratio per pixel, 1–1.5 hr after injection, in patients with systemic vasculitis was 0.87 (s.d. 0.25), significantly higher ($p < 0.001$) than the ratio 0.38 (0.13) in patient controls who had normal white cell scans. The majority of patients with systemic vasculitis had scintigraphic evidence of abnormal splenic function. Two had focal splenic defects, while 7 had increased labeled cell uptake. Nine of the patients with vasculitis showed cell migration into the gut, presumably as a result of vasculitis, and in 6 it was prominent. Focal nasal uptake was found in 5/7 patients with systemic vasculitis who had their heads imaged, and may be specific for WG. Although all patients had renal disease, there was scintigraphic evidence of diffuse parenchymal renal uptake of ^{111}In-labeled granulocytes in only one (with MP). The presence of anti-neutrophil cytoplasmic antibodies did not correlate with any abnormality or with lung uptake. Systemic vasculitis is associated with abnormalities of granulocyte kinetics, particularly involving the lung and spleen.

J Nucl Med 1992; 33:491–497

Increased pulmonary granulocyte margination, as a result of activation in vivo by certain cytokines, is thought to occur in several clinical settings, including extracorporeal circulation (*1*) and in the adult respiratory distress syndrome (ARDS) (*2*). Following injection of ^{111}In-labeled granulocytes, abnormal focal activity has been recorded in the lungs of patients with systemic vasculitis, in particular Wegener's granulomatosis (WG) (*3*). We have previously seen increased diffuse activity in the lungs, not accompanied by abnormal focal activity, in patients with systemic vasculitis, including microscopic polyarteritis (MP), during the course of routine, clinically indicated, ^{111}In-granulocyte scanning, suggesting that WG and MP may both be associated with increased pulmonary granulocyte margination. Patients with WG or MP have circulating antibodies to components of neutrophil cytoplasm (ANCA) which are strongly associated with active disease and which activate granulocytes in vitro (*4*). Anti-endothelial cell antibodies have also been demonstrated in systemic vasculitis (*5*). Activation of granulocytes in vivo by ANCA and/or of up-regulation of pulmonary endothelial leukocyte adhesion molecules (*6*) might lead to increased margination in the pulmonary vasculature in systemic vasculitis. In order to investigate this possibility, we have retrospectively semiquantified radiolabeled granulocyte activity in the lungs of patients with systemic vasculitis and correlated it with ANCA status.

Received Jul. 30, 1991; revision accepted Oct. 16, 1991.

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METHODS

Patients

The patients in this retrospective study were recruited from referrals to the Nuclear Medicine Unit for routine leukocyte scanning. The group was comprised of 12 patients with systemic vasculitis (WG = 6; MP = 6) and 28 control patients. All vasculitis patients in this study were diagnosed according to previously defined clinicopathological criteria (*7,8*). All had small-vessel vasculitis; patients with WG had evidence of granulomata and had prominent involvement of the upper respiratory tract. The clinical indications for the leukocyte scan in the patients with systemic vasculitis were generally either for the evaluation of suspected gut vasculitis or for unexplained pyrexia. Their clinical details are summarized in Table 1. All had renal disease and all were receiving treatment at the time of their scan with a standard immunosuppressive regimen which comprised prednisolone and cyclophosphamide (*9*). One patient with MP had abnormal liver function tests. Two patients (one with WG and one with MP) were studied twice at different stages of their disease. All of the control patients had unequivocally negative leukocyte scans and no evidence of inflammatory disease on follow-up to date. Twenty-two had irritable bowel syndrome, two had painful knees, one had bone pain several years after gunshot wounds, one had diarrhea following radiotherapy, one had pyrexia following a

TABLE 1
Clinical Summary (At Presentation)

Patient	Diagnosis	ANCA	ENT disease	Chest: Focal disease	Chest: Hemorrhage	Chest: Other	Gut symptoms	LFT	Interval: presentation to scan (days)	Indication for scan
DD	MP	+	No	−	+		No	N	79	pyrexia
NJ	MP	NA	No	−	−	COAD	Yes	N	16	gut disease
GC	MP	NA	No	−	−		Yes	N	27	gut disease
VB (1)	MP	+	No	−	+		Yes	N	19	pyrexia
VB (2)	MP								26	pelvic abscess
CO	MP	+	No	−	−		Yes	N	14	pyrexia,?ENT
DC	MP	+	No	−	−	basal atelectasis	Yes	Abnormal	15	pyrexia
HS (1)	WG	+	No	+	+		Yes	N	19	pyrexia,?gut
HS (2)	WG								64	pyrexia
EF	WG	+	No	+	−		No	N	17	septic knee
GJ	WG	+	Yes	−	−	pleural effusion (left)	No	N	76	pyrexia
MA	WG	+	Yes	+	+		Yes	N	63	gut disease
AM	WG	+	No	+	+		No	N	29	evaluation of focal lung pathology
DN	WG	+	No	+	−		No	N	8	pyrexia

NA = not known; MP = microscopic polyarteritis; WG = Wegener's granulomatosis; and COAD = chronic obstructive airway disease.
+ present, − absent.
All patients had renal disease.

minor gynecological procedure and in one it has not been possible to obtain a final diagnosis. The control group did not include any patients with inflammatory bowel disease or any splenectomized individuals. None of the controls was known to have underlying liver or lung disease. Anti-neutrophil cytoplasmic antibodies (ANCA) were detected by the internationally standardized indirect IF method (*10*).

Radiolabeled Neutrophil Studies

Radiolabeled white cell studies were performed with ^{99m}Tc-HMPAO (*11*) or ^{111}In tropolonate (*12*). Cells were labeled using standard techniques (*11,12*). Technetium-99m-HMPAO was used in 10 of the controls and in five of the WG/MP patients (Table 2). Of the two patients studied twice, one had MP and the other had WG. In each of these two patients, the follow-up study was with a different radionuclide compared with the first (Table 2).

Since these were routine referrals, multiple spot views were obtained, with a wide field of view gamma camera, over the whole body, including posterior views of the chest, between 1 and 1.5 hr after injection (very early views), then between 2.5 and 4 hr (early views) and finally at 20-24 hr (late views).

TABLE 2
Scintigraphic Features

Patient	Diagnosis	Cell prep	ANCA*	Lung uptake: Diffuse	Lung uptake: Focal	Nasal uptake	Gut uptake	Spleen	Other
DD	MP	Tc	+	−	NA	NA	+	enlarged	
NJ	MP	In	NA	−	−	NA	++	hot	
GC	MP	In	NA	−	NA	NA	++	enlarged	diffuse renal uptake
VB (1)	MP	In	±	++	−	+	++	normal	
VB (2)	MP	Tc	±	++	−	+	++	normal	
CO	MP	In	+	±	−	−	+	moderately hot	
DC	MP	In	+	+	−	NA	++	normal	
HS (1)	WG	Tc	+	++	−	+	+	focal defects	
HS (2)	WG	In	−	++	−	+	−	focal defects	
EF	WG	In	+	+	−	++	++	enlarged, hot	uptake in knee
GJ	WG	Tc	−	++	−	−	++	enlarged, hot	
MA	WG	Tc	−	++	+	NA	+	hot	
AM	WG	In	−	+	+	+	−	focal defects	
DN	WG	In	+	+	−	+	−	normal	

NA = not available or not imaged; MP = microscopic polyarteritis; WG = Wegener's granulomatosis.
++ marked, + present, − absent.
* At time of scan.

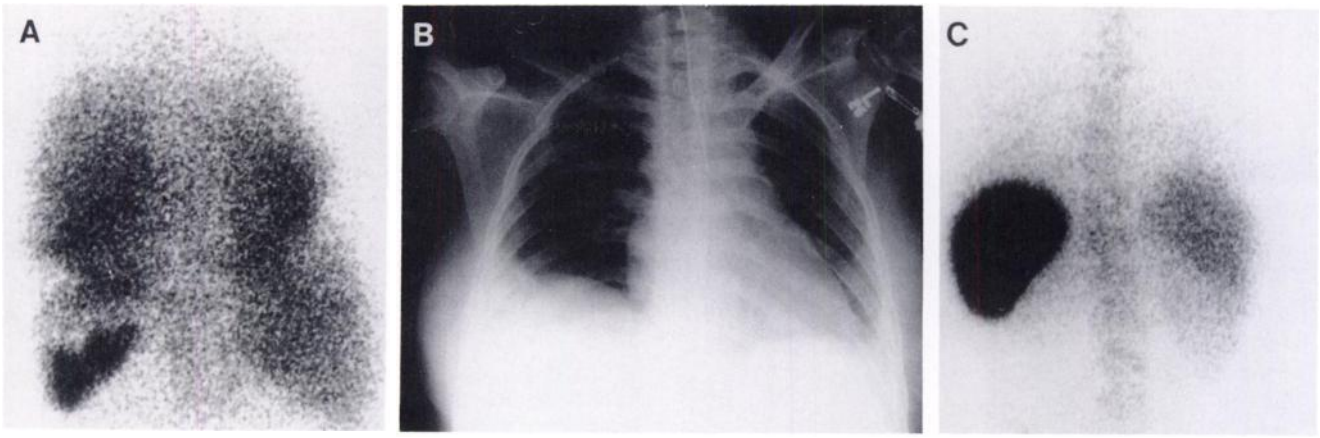

FIGURE 1. (A) Posterior chest view, showing increased diffuse lung activity (lung:liver ratio, 1.08) 1 hr following injection of ^{99m}Tc-HMPAO labeled leukocytes in a patient with Wegener's granulomatosis (HS, study 1). Note also the focal splenic defects. (B) Chest x-ray, showing a right-sided pleural effusion and a smaller one on the left, but clear lung fields. Note the presence of a nasogastric tube. (C) A patient from the control group given ^{99m}Tc-labeled cells: same gamma camera view and same time after injection (lung:liver ratio, 0.34).

Analysis

Regions of interest (ROIs) were drawn over the right lung, spleen and liver from the posterior aspect. The count rate in the right lung (per pixel) was expressed as a quotient of the count rate per pixel in the liver and in the spleen. In two patients with WG there was abnormal focal uptake in the left lung, which was not included in the lung ROI. Spleen:liver count ratios (per pixel) were also calculated.

Statistics

This was a retrospective study and so in some studies the appropriate views were not available. Specifically, these were the very early views in four studies in MP and one in WG, the early views in one study in MP and one in WG, the late views in two ^{111}In studies (one MP and one WG), and views of the face in four MP studies and one WG study (Table 2). Patient and study numbers therefore show some variation from one measurement/observation to another. Parametric statistics have been employed, using the Student t-test for unpaired data.

RESULTS

Abnormal Findings in Systemic Vasculitis

All patients with WG had visibly increased diffuse lung activity on the very early and early images, which had largely cleared on the late images (Fig. 1). Diffuse lung activity was generally less impressive in MP, although in three studies (two in the same patient) it was visibly increased, again clearing by 24 hr. In two patients with WG, a single abnormal focus of activity was seen in the left lung. In each case, this was superimposed on diffusely increased activity but, in contrast to the diffusely increased activity, became more prominent on the late views.

In seven patients with systemic vasculitis images were obtained of the face. Increased activity in the nasal region was seen in five, four of whom had WG (Fig. 2). Two of the seven had repeat studies and abnormal nasal activity was again seen in both. Nasal uptake did not correspond exactly to a clinical diagnosis of WG or to clinical evidence of active disease in the nose: uptake was seen in four patients with WG (of whom three had clinical disease in the nose) and in one patient with MP. However, this patient with MP had a nasogastric tube in situ in the period leading up to *both* of his studies. Two of the patients with WG showing nasal uptake also had nasogastric tubes, although in one of these, studied twice, only prior to the first study. One patient with WG and clinicopathological evidence of disease in the nose had no nasal uptake.

One patient with rapidly progressive glomerulonephritis and acute renal failure showed faint abnormal diffuse uptake of ^{111}In-labeled granulocytes in the parenchyma of the kidneys (Fig. 3).

All patients with MP and four with WG had abnormal uptake in the gut, consistent with vasculitis (Fig. 4). Of these, 5/6 patients with MP and 2/4 with WG had symptoms compatible with gut vasculitis and one in each group had vasculitic changes demonstrated at laparotomy. When imaged with ^{99m}Tc-HMPAO, this abnormal gut activity was visible within 1 hr of cell injection (i.e., it was not the result of the nonspecific bowel activity seen with this agent). When imaged with ^{111}In, it was visible within 3 hr

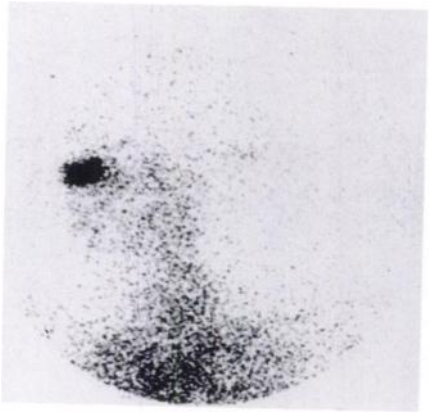

FIGURE 2. Increased nasal uptake (left lateral view) seen 20 hr after injection of ^{111}In-labeled granulocytes in a patient (EF) with Wegener's granulomatosis.

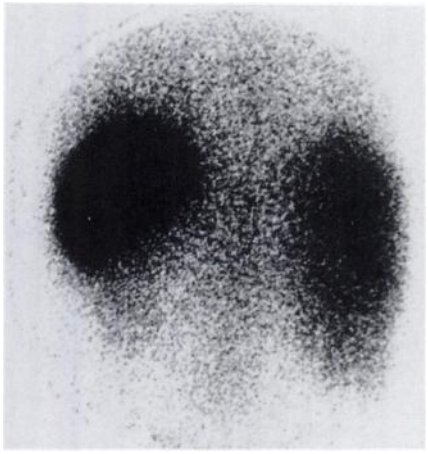

FIGURE 3. Posterior abdominal view, showing diffuse abnormal bilateral renal parenchymal uptake 8 hr after injection of ^{111}In-labeled granulocytes in a patient (GC) with microscopic polyarteritis.

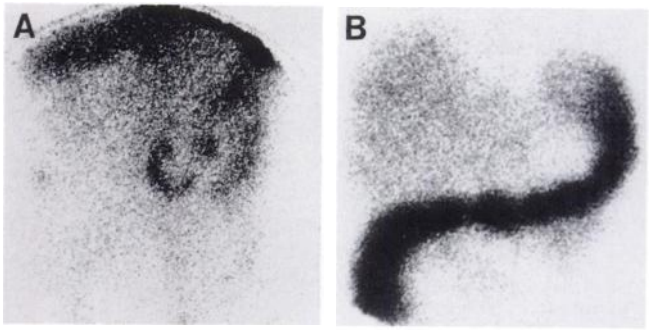

FIGURE 4. (A) Anterior abdominal view showing abnormal uptake of ^{111}In-labeled granulocytes in a loop of small bowel and the colon at the splenic flexure 1 hr after injection of ^{111}In-labeled granulocytes in a patient (DC) with microscopic polyarteritis. (B) Anterior abdominal view at 24 hr shows evidence of distal transit of small bowel activity, reaching ascending and transverse colons.

(i.e., was not due to swallowed activity). One patient with WG who was ANCA-positive and had abnormal gut activity consistent with gut vasculitis (with ^{99m}Tc) had no evidence of gut uptake when restudied (with ^{111}In) at the time she was ANCA-negative, but still had diffusely increased lung activity.

There was a high incidence of abnormal splenic uptake in the systemic vasculitis group. Two patients with WG (one studied twice) had multiple focal splenic defects on scintigraphy (Fig. 1). In seven further studies, the spleen was either enlarged or appeared unusually "hot" (or both) (Fig. 5). The spleen looked normal in only three patients (one of whom was studied twice).

Quantitative Comparison of Systemic Vasculitis with Controls

There was no significant difference in the lung:liver ratio between controls given ^{111}In-labeled cells and controls given ^{99m}Tc-labeled cells. The lung:liver, lung:spleen and spleen:liver ratios for ^{111}In were therefore each pooled with the corresponding ratios based on ^{99m}Tc in both groups of patients.

The mean lung:liver ratios per pixel in the control patients were 0.38 (s.d., 0.13, n = 18), 0.32 (0.13, n = 28) and 0.16 (0.06, n = 16 [^{111}In only]) on the very early, early, and late views, respectively. Taking systemic vasculitis as one group, the lung:liver count ratios were clearly elevated on the very early and early views: 0.87 (0.25, n = 9, $p < 0.001$) and 0.75 (0.26, n = 12, $p < 0.001$), respectively (Fig. 6). The mean ratio on the late views in this group was 0.27 (0.06, n = 7 [^{111}In only], $p < 0.002$), also significantly higher than the mean ratio in the late views in the control patients. The mean lung:liver ratio on the early views was significantly higher in WG as compared with MP: 0.94 (0.23, n = 6) versus 0.57 (0.22, n = 6, $p < 0.01$), respectively (Fig. 6). (Insufficient numbers were available for the two other imaging times.) Nevertheless, the ratio in MP was still significantly higher than the controls on the early views ($p < 0.001$).

Spleen:liver count ratios per pixel in the controls were 4.1 (2.2, n = 17) and 4.0 (1.8, n = 24) on the very early and early views, respectively. Excluding the two patients

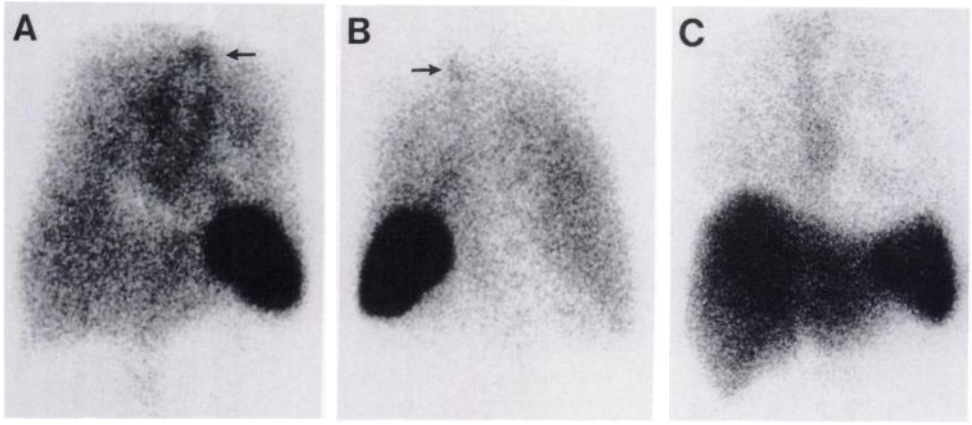

FIGURE 5. Intense uptake of ^{99m}Tc-HMPAO-labeled leukocytes in a normal-sized spleen (spleen:liver ratio, 9.3) 1.5 hr after injection in a patient (MA) with Wegener's granulomatosis. Note the intensity on the anterior view (A), in which the spleen, because of its posterior location, is not normally so prominent. There is also an area of focal uptake in the left apex (arrowed), visble on the anterior and posterior (B) views and superimposed on abnormally increased diffuse lung activity (lung:liver ratio, 1.08). An anterior view in a patient from the control group given ^{99m}Tc-labeled cells (C) is shown for comparison with (A).

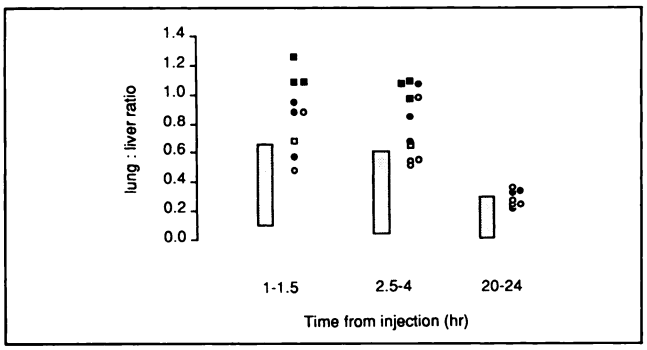

FIGURE 6. Comparison of lung:liver count rate ratio (per pixel) between systemic vasculitis and control patients, and, within the systemic vasculitis group, between Wegener's granulomatosis (closed symbols) and microscopic polyarteritis (open symbols). Values based on ^{111}In are shown as circles and on ^{99m}Tc as squares. The 95% confidence intervals for the control patients are shown as stippled bars.

with splenic defects, the sleen:liver ratios in systemic vasculitis were significantly higher at both these imaging times: 6.8 (4.1, n = 6, p = 0.05) and 7.2 (2.8, n = 8, p < 0.001).

Lung:spleen count ratios per pixel in the controls were 0.11 (0.045, n = 17) and 0.081 (0.024, n = 24) on very early and early views. After excluding the two patients with focal splenic defects, lung:spleen count ratios in systemic vasculitis were also higher than those in controls in the very early and early views: 0.16 (0.077, n = 6, 0.1 > p > 0.05) and 0.12 (0.067, n = 8, p < 0.05), respectively. Lung:spleen ratios were considerably more variable than the lung:liver ratios. There were insufficient numbers to compare lung:spleen ratios in WG with controls.

Presence or absence of ANCA at the time of scintigraphy did not appear to correlate with any of the above ratios, or with the presence or severity of gut involvement or splenic abnormalities.

DISCUSSION

It is widely believed that most of the MGP is in the lung (*13,14*), although this has not been borne out with the advent of clinical white cell scanning for the diagnosis of inflammatory disease (*15*). Our own estimate of the lung MGP, based on kinetic studies and a comparison of ^{111}In-labeled neutrophils with radiolabeled red cells (*16,17*), is that it represents only about 10% of the whole-body MGP; i.e., neutrophils normally show no more tendency to marginate in the lungs than the overall average for the whole body (*18,19*). This estimate is consistent with the changes observed in the lung ^{111}In granulocyte signal following exercise, which in our hands did not produce any pulmonary demargination (but did result in marked splenic demargination) (*20*), and inhalation of platelet activating factor (PAF), which resulted in an acute doubling of the lung^{111}In granulocyte signal (*21*).

Increased, pathophysiological, pulmonary granulocyte uptake must be distinguished from the immediate lung signal which is observed when granulocytes have become activated as a result of manipulation in vitro during labeling (*15*). A characteristic feature of this "artifactual" lung sequestration is that the lungs become clear by about 40 min, as the labeled neutrophils move from the lung into the reticuloendothelial system, particularly the liver. As a result, by 1 hr, the lung:liver ratio is very low (*15*). The recovery of labeled cells in blood (i.e., the percentage of injected labeled granulocytes still circulating in blood 30 min after injection) is also low and radioactivity fails to localize in sites of inflammation. This artifactual lung sequestration is almost completely abolished when granulocytes are isolated on density gradient columns made up with autologous plasma or when "mixed" leukocytes are isolated and labeled in plasma (*15*). We have previously shown similar early patterns of biodistribution for ^{111}In-tropolonate-labeled granulocytes and ^{99m}Tc-HMPAO-labeled leukocytes (both preparations labeled in plasma), in particular similar initial rapid lung transit and minimal localization in the liver (*22*). The characteristic feature of genuine pathophysiological margination in the lung is an intensity of uptake which is broadly proportional to the level of circulating granulocyte activity and, unlike artifactual lung hold up, is not associated with a high liver uptake.

Increased pulmonary granulocyte margination could result from granulocyte activation in vivo, resulting in increased "stiffness" or "stickiness," or "up-regulation" of leukocyte adhesion receptors on the pulmonary endothelium (*6*). Granulocytes could become activated in vivo as a result of exposure to ANCA. However, this appears unlikely since an increased lung radioactivity signal was still present in patients who were ANCA-negative at the time of scintigraphy. Exposure to cytokines at a site of inflammation is another possible cause of granulocyte activation in vivo since cytokines increase the stiffness of granulocytes and prolong their passage through the lungs (*23*). This mechanism has been proposed in Crohn's disease (*24*) where granulocytes may be exposed to cytokines as they pass through inflamed gut without leaving the intravascular space. Granulocytes from patients with Crohn's disease show increased adherence in vitro (*24*) and this fits with the increase in lung:liver ratio that we have previously recorded in inflammatory bowel disease (IBD) (*25*). However, although most of the patients with systemic vasculitis in this study had clinical evidence of gut vasculitis with migration of labeled granulocytes into the gut wall and bowel lumen, as in IBD (*26*), it is unlikely that the increased pulmonary granulocyte margination of systemic vasculitis is secondary to gut vasculitis. Hence the lung:liver ratio did not correlate with the intensity of gut uptake and was generally much higher than those recorded in severe IBD (*25*). This, however, does not exclude other, as yet unrecognized, causes of granulocyte stiffness or stickiness in systemic vasculitis.

Increased margination may be due to properties of the

endothelium, for example, up-regulation of adhesion molecules (*6*). This could also be induced by cytokines. Interleukin-1, for instance, induces marked granulocyte accumulation in the lungs of experimental animals (*27*), while in man we have shown that inhalation of PAF causes an immediate increase in pulmonary granulocyte margination (*21*). Alternatively, margination may be increased by an, as yet undefined, effect of the anti-endothelial antibodies which have been detected in the sera of some patients with systemic vasculitis (*5*).

Immunosuppressive therapy administered to these patients seems an unlikely cause of the increased granulocyte margination. Steroids would, if anything, reduce it (*28*), and although WG and MP were treated essentially the same, there was a significantly higher lung signal in WG.

Although the lungs appeared largely to clear by 24 hr in all patients with a diffusely increased uptake in the early views, the lung:liver ratio remained significantly elevated in the patients with systemic vasculitis. In addition to increased focal lung activity, which was seen in only two patients, there could be some degree of diffuse migration of granulocytes in the lungs in systemic vasculitis in addition to, and possibly resulting from, the increased margination. In general, it is not clear from granulocyte kinetic studies if a stimulus to increased margination invariably leads to some degree of granulocyte migration.

The intensity of splenic activity, although highly variable in WG and MP, was clearly increased in some patients, and this was an unexpected finding. It is rather difficult to interpret but may be due to increased splenic blood flow or a prolonged granulocyte intrasplenic transit time, which could result from increased stickiness of granulocytes and/or increased adhesiveness of endothelial cells, as in the lung. This intense splenic uptake is reminiscent of increased splenic platelet uptake which we have previously seen in patients with various connective tissue disorders, but without splenomegaly, and which is the result of a markedly increased splenic blood flow (*29*). The variability of splenic uptake is not surprising in view of the wide variation in splenic shape and size and, in some patients, the presence of focal defects of splenic uptake. The range of splenic appearances in these patients suggests that in systemic vasculitis there may be a progression from increased splenic blood flow, followed by splenic enlargement and/or splenic infarction resulting from increased blood cell input into the spleen. Splenic abnormalities in WG have been described on CT as hypodense areas (*30*) but the relationship of these to the scintigraphic abnormalities reported here is not clear. As an alternative to infarction, the latter may represent splenic granulomata.

These studies have demonstrated focal abnormalities in the lung, gut, and nasal region which broadly correspond to sites of clinical disease activity. Thus leukocyte scintigraphy may have a diagnostic role in the evaluation and extent of vasculitis. Nasal uptake may be specific for WG, which is also associated with a more prominently abnormal pulmonary granulocyte signal. With the increasing interest in the role of neutrophils in lung injury and systemic organ failure (*27,31*), the observation of diffusely increased lung granulocyte accumulation, consistent with increased pulmonary margination, is also important.

In conclusion, we have presented evidence of increased granulocyte margination in the lungs in systemic vasculitis, especially WG. It will now be important to: (1) strengthen these observations with a more rigorous quantification of pulmonary margination in vivo, such as the simultaneous use of radiolabeled red cells as a marker for the circulating component of the lung granulocyte signal and against which the transit time of the labeled granulocyte could be compared (*17*); and (2) identify whether the granulocyte or pulmonary endothelium is primarily activated. Furthermore, patients with systemic vasculitis sometimes have abnormalities of the spleen, which may collectively be expressed as splenic "vasculitis."

ACKNOWLEDGMENTS

GG is a Medical Research Council training fellow.

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ERRATUM

In the January issue of the *Journal*, references 14–20 were omitted in the article, "Cyclic Oral Phosphate and Etidronate Increase Femoral and Lumbar Bone Mineral Density and Reduce Lumbar Spine Fracture Rate Over Three Years," by Silberstein and Schnur. The references are printed below.

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