Comparison of Purified Indium-111 Granulocytes and Indium-111 Mixed Leukocytes for Imaging of Infections

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Several methods have been proposed for the separation and labeling of white blood cells for the diagnosis of suspected infection. We retrospectively compared 105 patients imaged with ^{111}In purified granulocytes (GRAN) to 106 patients imaged with ^{111}In mixed leukocytes (MIX). We found that in acute infection the sensitivity of GRAN and MIX were both high and not statistically different. In chronic infections the sensitivities were lower than for acute infections. Again, there was no significant difference between GRAN and MIX with the borderline significant exception of MIX being superior to GRAN in chronic soft tissue infections ($p = 0.06$). We then had independent observers blindly grade the degree of lesion visualization. We found that delayed images visualized the lesions better than early images ($p = 0.0001$) and acute infection was better visualized than chronic infection ($p = 0.03$). We concluded that, in routine clinical practice, MIX is probably the agent of choice for three reasons: (a) easier preparation, (b) comparable sensitivity in acute infection and, (c) borderline superior sensitivity in chronic infection.

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Several methods have been proposed for the separation and labeling of white blood cells for the detection of soft-tissue infections and osteomyelitis. Thakur has proposed an augmented sedimentation procedure that provides a mixture of leukocytes for labeling (*1*). This technique has been widely used for routine clinical studies (*2–5*). The chief advantage is that it is easier to perform than the more complex procedures required to separate individual cell types. Other groups have used the more complex separation procedures to obtain purified granulocytes (*6–8*).

In this study we compared the advantages and disadvantages of purified indium-111 (^{111}In) granulocytes (GRAN) with ^{111}In-labeled mixed leukocytes (MIX). In all cases the cells were labeled with [^{111}In]tropolone in a plasma environment and never exposed to saline. The cell preparations were as similar as possible in an effort to minimize any differences, except for those caused by the cell types studied.

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We specifically wished to answer the following questions

1. Is there a difference in sensitivity using GRAN or MIX in either acute or chronic infection?
2. Does GRAN or MIX visualize the site of infection better?

MATERIALS AND METHODS

Patient Population

Between 10/11/83 and 2/25/85, 105 patients (70 M, 35 F), aged 16–80 yr (mean 47 yr) were studied with [^{111}In]GRAN. Sixty of these patients had pathologically proven diagnoses. Forty-five patients had clinical diagnoses and long-term follow-up. Between 3/4/85 and 1/3/86, 106 patients (77 M, 29 F), aged 18–83 yr (mean 51 yr) were studied with [^{111}In]MIX. Sixty-one of these patients had pathologically proven diagnosis and 45 patients had clinical diagnosis and long-term follow-up. The patient population all came from a large general hospital and from a large Veterans Hospital. The patient population appeared stable during the duration of this study.

Cell Separation and Labeling

Our cell separation and labeling procedure is a modification of the discontinuous metrizamide-plasma (MP) gradient pro-

TABLE 1
Scale for Lesion Visualization Score

Score	Description
0	No abnormality seen
1	Questionable localization
2	Faint, but definite localization
3	Moderate localization
4	Intense localization

cedure of Saverymuttu et al. (*9*), and has been described in detail elsewhere (*8*). In short, 43 ml of whole blood is collected in a syringe containing 7 ml of ACD and 10 ml of 6% hydroxyethyl starch. Following red blood cell sedimentation, leukocyte-rich plasma is obtained and softly centrifuged (100 $g \times 10$ min), giving a mixed leukocyte pellet and platelet-rich plasma. The mixed leukocyte pellet is resuspended in platelet-poor plasma and underlayed with a 40% v/v gradient and a 47% v/v MP gradient. Following hard centrifugation (2,000 *g* for 10 min), mononuclear leukocytes (monocytes, lymphocytes) and residual platelets are found at the plasma: 40% MP interface, and granulocytes (> 95% neutrophils) are found at the 40%–47% MP gradient interface with variable red cell contamination (0%–30%).

Indium-111 GRAN was prepared by harvesting only the granulocytic layer and labeling in plasma with freshly prepared [^{111}In]tropolone. Following a plasma wash, < 5% of the injected radioactivity was red blood cell bound, whereas, the remainder was granulocyte bound (> 95% neutrophils). For the purpose of maintaining nearly identical handling effects, [^{111}In]MIX was prepared by harvesting, and then combining both the mononuclear leukocyte and granulocyte layers. Labeling of MIX with [^{111}In]tropolone was performed exactly the same as for [^{111}In]GRAN. Because no leukocytes were eliminated by this separation and recombining technique, the labeled leukocyte differential paralleled each patient's white blood cell differential. No attempt was made to quantify the percent of radioactivity bound to each specific leukocyte type.

Double-Blind Image Evaluation

Each patient had early (~4 hr) and delayed (~24 hr) images. The images for a given patient were grouped and separated according to time after reinjection of the ^{111}In preparation. These images were then coded and placed on viewboxes in random fashion. Independently, three experienced nuclear medicine physicians blindly read and scored the scan results according to the criteria of Table 1.

Sensitivity and Specificity Comparisons

In 121 cases the "correct" diagnoses were provided by surgical pathology or by biopsy and culture; in the remaining 90 cases the discharge diagnosis and clinical follow-up, when available, were considered the "correct" diagnosis. The differentiation of acute and chronic infection was based on the pathologists report when available or upon an arbitrary 2-mo duration of symptoms for the patients followed clinically. These two groups of patients were combined giving the 211 total patients which are evaluated in this study. The studies were read as positive when the visualization score was ≥2 (Table 1).

RESULTS

Table 2 contains the number of patients with true positive results in acute osteomyelitis (AOM), chronic osteomyelitis (COM), acute soft-tissue infection (AST) and chronic soft tissue infection (CST), and the true negative studies for both GRAN and MIX. In addition, the sensitivity and specificity is given in parenthesis below the numbered patients in Table 2. Statistical analyses employed a 2 × 2 contingency table. Fisher's exact probability test was used to state the results. The sensitivity of MIX is borderline superior to GRAN for CST infections ($p = 0.06$). When studying GRAN alone, sensitivity in AST is higher than for CST ($p = 0.002$) and the sensitivity for AOM is not significantly higher than for COM. For MIX there was no significant difference in any of the comparisons of acute versus chronic infections.

The mean visualization scores and range of values for GRAN and MIX are given in Table 3. Because the mean of the scores of the three observers was used for

TABLE 2
Results in All Cases

	True positives (Sensitivity)				True negatives (Specificity)
	AOM*	COM†	AST‡	CST§	
Indium-111 GRAN	11/11 (100%)	19/24 (79%)	15/15 (100%)	7/15 (47%)	37/40 (92%)
Indium-111 MIX	7/8 (88%)	28/33 (85%)	17/17 (100%)	11/13 (85%)	32/35 (92%)

* AOM = Acute osteomyelitis.
† COM = Chronic osteomyelitis.
‡ AST = Acute soft-tissue infection.
§ CST = Chronic soft-tissue infection.

TABLE 3
Mean and (Range) of the Visualization Scores

Images	Agent	AOM*	COM†	AST‡	CST§
Early	GRAN	2.94 (1.0–4.0)	2.62 (0.0–4.0)	2.02 (0.0–4.0)	1.82 (0.0–3.3)
	MIX	1.90 (0.33–4.0)	2.13 (0.0–4.0)	2.84 (0.0–4.0)	1.66 (0.33–4.0)
Delayed	GRAN	3.15 (1.7–4.0)	2.92 (0.0–4.0)	2.94 (0.0–4.0)	2.43 (1.0–4.0)
	MIX	2.80 (0.33–4.0)	2.57 (0.0–4.0)	3.31 (0.0–4.0)	2.18 (0.33–4.0)

* AOM = Acute osteomyelitis.
† COM = Chronic osteomyelitis.
‡ AST = Acute soft tissues.
§ CST = Chronic soft tissues.

each patient, statistical analysis was based on the analysis of variance and the Dunn–Sidak method for multiple comparison.

The results from the analysis of variance revealed two main effects that were statistically significant: (a) The delayed images gave better visualization of the lesion than did the early images ($p = 0.0001$) and, (b) acute infections are better visualized than chronic infections ($p = 0.03$). None of the interactions between any two or more factors was significant.

DISCUSSION

GRAN is less sensitive in CST than for AST ($p = 0.002$) and borderline less sensitive, though not significant, for COM than AOM. For MIX there was no statistically significant difference in any of the comparisons of acute versus chronic infections. This decreased sensitivity of GRAN for chronic infections may be the reason that we found MIX to be borderline significantly more sensitive than GRAN for detecting CST infections ($p = 0.06$). Labeled lymphocytes in the MIX preparation, which were intentionally excluded in the GRAN preparation, may explain these findings. As the infection progresses from an acute to chronic stage, the cellular response changes from a predominently granulocytic response to a predominently lymphocytic response (*10*). Goodwin has previously shown the value of the labeled lymphocyte in five patients when the labeled granulocytes failed to visualize the chronic infection, which was correctly identified with labeled lymphocytes (*11*).

The analysis variance of the visualization scores (Table 3) revealed two statistically significant findings: (a) The delayed images gave better visualization of the lesion than did the early images ($p = 0.0001$) and, (b) acute infections are better visualized than chronic infections ($p = 0.03$). Of equal importance are the comparisons that were not statistically significant. First, there was no significant difference between the visualization of osteomyelitis and soft-tissue infections. Second, there was no significant difference in GRAN and MIX in visualizing the site of infections.

For routine clinical work MIX appears to be the agent of choice for two reasons: (a) the preparation is significantly easier, particularly if one follows the standard Thakur procedure (*1*) and, (b) in no case was GRAN significantly better than MIX; however, MIX is borderline significantly better than GRAN for chronic infections due to the presence of labeled lymphocytes.

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