Letters to the Editor

Lymphocyte Radiolabeling: A Challenge to Their Survival

TO THE EDITOR: In a recent paper, Merz et al. described the detrimental effects induced by cell labeling with technetium-99m (^{99m}Tc) on genetic material of lymphocytes (*1*). They clearly demonstrated that, even with a labeling activity as low as 240 μCi/10^7 cells, the labeling procedure induced a loss of proliferative capacity and damage to genetic material, with no cells reaching the second division. However, they conclude that lymphocytes ^{99m}Tc labeling compares favorably with the widely accepted labeling technique of lymphocytes with indium-111 (^{111}In) and they estimated the dose received by the cells to 60 cGy. Although this study points out once again the radiotoxicity of cell labeling on the most radiosensitive cells, lymphocytes, the author's conclusion and dosimetry lead us to make some remarks.

Merz's estimation of the dose received by lymphocytes during ^{99m}Tc labeling is supported by Ten Berge (*2*) using lymphocytes labeled with ^{111}In. Ten Berge's estimation of the dose received by lymphocytes labeled with 9 μCi ^{111}In/10^7 cell (2 to 2.5 Gy) relied on the fact that the chromosomal abnormalities he observed were close to those noticed by Buul when the same dosage is delivered to the cells by an external irradiation using 240 keV (*3*). From Ten Berge's estimation and his own results, Merz concluded that human lymphocytes labeled with ^{99m}Tc in a concentration of 242 μCi/10^7 cells has a 20% loss of proliferative capacities and damage to genetic material approximating an exposure of 60 cGy of 250 keV x-ray.

This conclusion looks rather crude for the following reasons.

1. To assess the viability of the cells, Merz used different biologic tests from those used by Ten Berge et al. (*2*). The latter assessed genetic damage after indium labeling by measuring directly the frequencies of chromosome and chromatic aberrations induced by the radiotracer and studied the proliferative capacity of lymphocytes by the incorporation of ^{3}H thymidine under several stimuli (*2*). Merz on the other hand studied indirectly the chromosomal damage by counting the number of micronuclei and estimated the division delay by mitotic index and Harlequin staining (*1*). For his estimation of the dose through the computation of Ten Berge he indirectly related his data to those of Buul who studied the genetic effects induced on lymphocytes by external irradiation with the common 250 keV standard (*3*). Still this author and Merz used different techniques to culture the cells. For instance, the cells used by Buul were not previously incubated by sodium pyrophosphate and stannous chloride. Merz used stimulated lymphocytes while Buul did not.

2. Furthermore, Buul on the basis on his results concluded that "there is not one human radiosensitivity to chromosomal aberration production in blood lymphocytes but that even amongst normal donor there exist strong differences" for the same radiation dosage. In view of the existence of immense variability with regard to the response it is not worth while to compare, as Merz did, his data with those of Ten Berge and indirectly Buul on the basis of tests studying genetic damage.

Therefore, Merz only can affirm that ^{99m}Tc lymphocytes labeling induces some degree of chromosomal aberrations only, but can hardly draw, at least from his results, some conclusions on the dose received by the cells. To achieve this aim it would be necessary to compare his results after ^{99m}Tc labeling to the results obtained using a common 250 keV x-ray standard or cobalt irradiation using lymphocytes in the same experimental conditions as we did (*4*).

Finally, we think that the evidence of genetic disorders which is the only effect studied by Merz does not imply anything on the homing properties of the cells. Lymphocytes with damaged chromosomes are still able to proliferate with quite normal DNA polymerase activity (*5*). On the other hand, the quality of homing is better estimated by the study of the properties of the cell surface. Using T cell markers and T cell functions we studied the effects of various activities of ^{99m}Tc labeling (*5*). By using activities two to three times higher than those used by Merz, we observed that E Rosette formation was reduced and that ^{3}H thymidine incorporation with PHA was greatly impaired. This suggested a destruction or an inhibition of the T cells membrane receptors for sheep erythrocytes and lectin. Allogenic cell mediated lympholysis was also studied and was found to be abnormal. We would have been interested to know what was the effect of Merz's technique on the results of these tests. In our study, morphological properties of the cells studied by transmission electron microscopy were unaffected, another result which shows that one cannot conclude at the lack of radiotoxicity looking at only one cell test (*4*).

Therefore, one must be very cautious when using ^{99m}Tc labeling of lymphocytes (*6*). We think it would be necessary before proceeding to any human studies to assess the function of lymphocytes labeled with low activity of ^{99m}Tc (as those used by Merz) with a battery of tests, particularly those exploring the properties of the cell surface. Otherwise from a physiological point of view, the injection of labeled lymphocytes would be a gamble with their survival.

References

1. Merz T, Tatum J, Hirsch J. Technetium-99m-labeled lymphocytes: a radiotoxicity study. *J Nucl Med* 1986; 27:105–110.
2. Ten Berge RJM, Natarajan AT, Hardeman MR, et al. Labeling with indium-111 has detrimental effects on human lymphocytes: concise communication. *J Nucl Med* 1983; 24:615–620.
3. Buul PPW, Natarajan AT. Chromosomal radiosensitivity of human lymphocytes in relation to sampling time. *Mutation Res* 1980; 70:61–69.
4. Meignan M, Charpentier B, Wirquin E, et al. Biological effects and irradiation dose induced in human lymphocytes in vitro by an intracellular radionuclide: 99m Tc. *Radiation Res* 1983; 94:263–279.

5. Agarwal SS, Katz EJ, Krishan A, et al. DNA replication in X-irradiated human lymphocytes. *Cancer Res* 1981; 41:3973–3978.
6. Parmentier C, Chavaudra J, Meignan M. Dose received by lymphocytes in the course of labelling techniques using 99m Tc. *J Nucl Med* 1978; 19:223.

Michel Meignan
Evelyne Wirquin
Université Paris XII
Hôpital Henri Mondor
Creteil, France

"Delta Sign" in Bone Scan Interpretation—A Cautionary Note

TO THE EDITOR: The "delta sign" has been described by Fink-Bennett and Vicuna-Rios (*1*) as a normal bone scan variant, representing the site of deltoid muscle insertion in the upper humerus. We recommend a degree of caution, however, in ascribing a focal increase in tracer activity to this normal variant, particularly in the presence of a known primary tumour which has the propensity for spread to bone, even if this is usually to the axial skeleton. We report the case of a 58-yr-old woman with a history of carcinoma of the cervix 13 yr previously, which had been treated by total abdominal hysterectomy preceded by radium insertion. She re-presented with a 2-mo history of a lump in the left breast. On examination a 5-cm mass was palpable in the upper outer quadrant of the left breast, with associated mobile axillary lymphadenopathy. As part of the staging procedure for the carcinoma technetium-99m methylene diphosphonate whole-body scintigraphy was performed. Only a single metabolically active lesion was noted which was in the upper third of the right humerus (Fig. 1). In the absence of lesions at other sites and the presence of a corresponding prominent deltoid tuberosity on the plain radiograph, the active lesion was ascribed to the "delta sign". A simple left mastectomy with axillary clearance was performed. At histology four of five lymph nodes in the upper axilla were involved with tumor and five of seven lymph nodes in the lower axilla were also infiltrated. In the presence of a negative bone scan the carcinoma was graded on clinicopathological grounds as a stage II tumor. A 6-wk course of radiotherapy was therefore given to the left chest wall and axilla 1 mo postoperatively.

Six months after her operation, the patient presented with an 8-wk history of tenderness in the right arm. A plain radiograph now showed a permeative bone lesion in the proximal third of the right humerus, suggestive of a metastasis. A repeat bone scan at this stage demonstrated a fusiform increase in tracer uptake in the upper third of the right humerus consistent with a metastasis (Fig. 2); the remainder of the skeleton being normal. The patient is currently receiving local radiotherapy with good relief of her symptoms.

The "delta sign" was described in 1980 and attributed to "the greater affinity of methylene diphosphonate for the cortically thickened site of insertion of the deltoid muscle". It was cited as a cause of localized increased tracer uptake in 7%

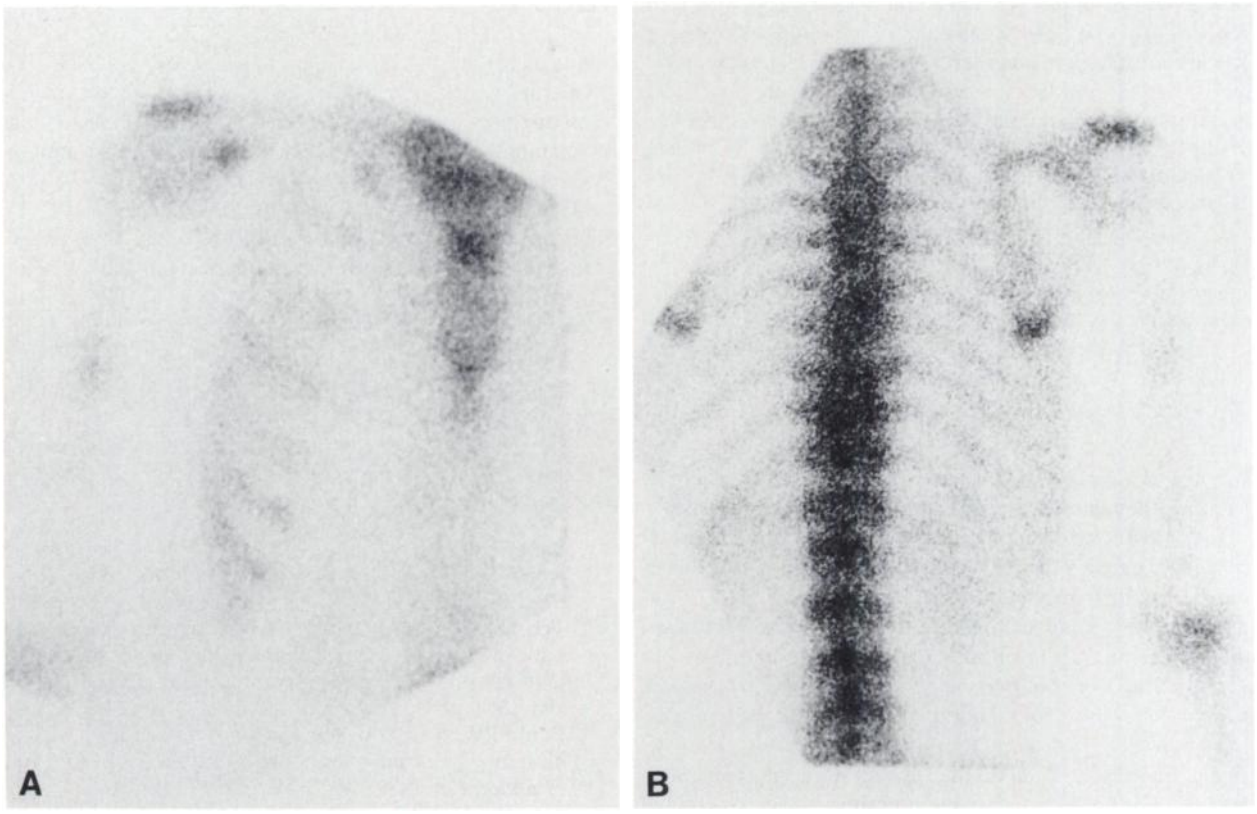

FIGURE 1
A: Anterior view of the right humerus. B: Posterior view of the right humerus. Note the focus of increased activity in the upper third of the right humerus, corresponding to the deltoid tuberosity on plain radiography.