Cell Labeling: Radiation Dose and Effects

For many years, nuclear medicine researchers have sought agents to successfully label cellular blood elements with radionuclides to determine the fate of the cells by both external imaging and in vitro measurements. As noted by ten Berge et al. in this issue, the label must meet the following requirements: The labeling procedure must have no effect on functional activities of the cells, and there must be little or no spontaneous release of the radioactive label (*1*). Several different radioactive labels (Cr-51, Tc-99m, In-111, and others) in combination with a number of different agents have been used. In 1976, McAfee and Thakur surveyed a variety of radioactive agents for in vitro labeling of phagocytic leukocytes and determined that the In-111 chelate of oxine showed promise as a suitable agent (*2*). Since that time, researchers have investigated this agent for cell labeling (*3–7*) and have also evaluated other radionuclides, e.g. Ru-97 (*7*). Newer coupling agents such as tropolone have been advocated to overcome some of the disadvantages encountered with In-111 oxine (*8*). Variations in cell labeling techniques have been designed to improve the characteristics of the product. These investigations have led to considerable discussion about the merits and limitations of the radionuclides and agents (*9–10*). The radionuclide with the most advantageous characteristics, the best agent, and the optimum labeling technique have not been identified, but concurrently an important consideration must be the radiation effects from the radionuclide.

Because of differences in the distributions of the blood elements in organs, the radiation-absorbed dose to the organs from the various types of labeled blood cells will differ. From studies on the distribution of In-111 labeled leukocytes and platelets in patients, Goodwin et al. estimated the dose from mixed leukocytes (predominantly neutrophils) labeled with 0.5 mCi (18 MBq) of In-111 to be 1.4 rad (14 mGy) to the liver, 8.5 rad (85 mGy) to the spleen, and 2.3 rad (23 mGy) to the bone marrow (*11*). Lymphocyte distribution was similar to that of mixed leukocytes with the exception that activity was found in the inguinal and cervical lymph nodes. The estimated dose from 0.5 mCi (18 mBq) of In-111 labeled lymphocytes was 0.8 rad (8 mGy) to the liver, 6.7 rad (67 mGy) to the spleen, and 1.4 rad (14 mGy) to the marrow and lymphatic tissue. Platelet studies showed that 40% to 60% of the activity was distributed to the blood pool, and that most of the remainder was concentrated in the spleen, with a small quantity in the penis. Goodwin et al. calculated the dose from 0.5 mCi (18 MBq) of In-111 labeled platelets to be 3.2 rad (32 mGy) to the liver, 8.6 rad (86 mGy) to the spleen, and 0.3 rad (3 mGy) to the total body.

Gaulden recently reported that conventional methods of calculating radiation dose may not be adequate to estimate radiation damage (*12*). She cited the work of Rao et al. who found that the biological effects in the mouse testis from the low-energy emissions of Tl-201 differed by a factor of four from those observed from the more energetic beta particles of Tl-204 per unit dose as calculated in the conventional manner (*13*). This situation is not unique to Tl-201 and the labeling of blood cells probably presents a similar matter of concern.

Even when human neutrophils and platelets receive a much higher radiation dose than that associated with standard procedures, investigators have found no apparent effect on these cells (*14–16*). Lymphocytes, however, have been shown to be extremely sensitive to radiation. According to Cronkite and Bond they are next in order of sensitivity to spermatogonia (*17*). For this reason, the article in this issue merits particular attention (*1*). The authors question whether labeling with In-111 oxinate can be used safely in vivo to monitor homing and recirculation of lymphocytes. They postulate that a transformed cell could proliferate into a malignant process. They also visualize the possibility of a similar hazard with the use of In-111 labeled granulocyte suspensions for detection of abcesses because of the presence of up to 20% lymphocytes in the suspensions.

These investigators are not the first to express the need for additional investigations to determine the potential for mutagenic and oncogenic effects from cell-labeling procedures. Parmentier et al. suggested that labeling lymphocytes with Tc-99m should be scrutinized carefully and that

efforts should be made to reduce the irradiation levels to which lymphocytes are exposed (*18*). They reminded investigators that Tc-99m not only emits gamma photons at 140 keV but also emits low-energy electrons. Indium-111, of course, also emits low-energy electrons, particularly Auger electrons (0.6 to 25.4 keV), which have an even shorter range in tissue.

In 1979, Frost and Frost expressed concern that the effects of the intrinsically high doses of radiation to these long-lived cells are unknown and advised restriction of the use of In-111 labeled lymphocytes to patients with shortened life expectancy (*19*). Goodwin (*20*) responded to Frost and Frost by referencing the comments of Rannie et al. on the results of Au-198 and Y-90 therapy in rheumatoid arthritis in which they reported that no neoplastic events had been seen (*21*). Rannie et al. also pointed out that 10^7 to 10^8 lymphocytes represent only 0.1% of the total recirculating lymphocyte pool, and because most of these cells are nondividing, the potential for oncogenesis is small. Although Goodwin did not feel as strongly as Frost and Frost did with respect to the use of In-111 to label lymphocytes he saw a need for carefully planned human studies to determine the effects of radiation dose on the viability of labeled cells.

Segal et al. reported on the damage to In-111 labeled lymphocytes by incorporated radioactivity and considered several aspects of the problem (*3*). Their study suggested that most of the lymphocytes would be killed if they were labeled with sufficient radioactivity to permit external detection. Although the next logical step would appear to be the modification of the labeling procedure to reduce radiation damage, the risk of mutagenesis would naturally increase because damaged long-lived cells with their potential for proliferation could survive.

In 1979, Chisholm et al. studied cell damage in rat lymphocytes and HeLa S3 cells labeled with In-111 oxine (*22*). At levels of 5 μCi (0.18 MBq) of In-111 oxine per 10^8 cells lymphocytes behaved normally; however, when the levels were increased to 10–40 μCi (0.37 to 1.5 mBq) aberrations in tissue distribution ranged from slight to severe. Lymphocytes labeled with 100–150 μCi (3.7 to 5.6 MBq) did not migrate normally in any instance. To determine whether the damage to cells was caused by the radiation from the decay of In-111, they treated cells with "indium-oxine" that had been allowed to decay. Their results suggested that their hypothesis was correct, and they felt that their findings might impose limitations on the use of In-111 oxine as a cell label for clinical purposes. Their results, which agreed with those of Segal et al. (*3*), indicated that the possible damage to cells should be investigated for each cell type considered for clinical tracer studies.

More recently Wagstaff et al. studied the distribution of radioactivity after reinjection of heat-damaged lymphocytes labeled with In-111 oxine (*23*). Although the distribution was quite different from that of "normal" labeled lymphocytes, their results did not indicate that the separation and labeling procedure was responsible for the significant damage to the lymphocytes. They recommended the specific lymphocyte activity be limited to 20 to 40 μCi (0.74 to 1.5 MBq) per 10^8 cells to minimize radiation damage.

The method used to estimate radiation-absorbed dose does not affect the radiation damage from a particular labeling procedure. It is simply a way of expressing the energy absorbed in terms that can be related to a biological response to the radiation. In an attempt to better describe this relationship, some investigators have estimated the radiation-absorbed dose to individual cells. In 1978 Goodwin concluded that each lymphocyte would contain 10^7 atoms of In-111/cell if 1.0 mCi (37 MBq) of In-111 were used for labeling (*24*). The dose to 10^6 lymphocytes labeled with 500 μCi (18 MBq) of In-111 to total decay would be 8.8×10^5 rad (8.8×10^5 Gy). Segal et al. estimated that the radiation dose rate per cell for a typical labeling procedure of 1 mCi (37 MBq) of In-111 per 10^8 cells would be 1700 rad/day (17 Gy/day) if the In-111 were at or near the center of the cell (*3*). They felt this estimate might be unrealistic, and a better value would be 1000 rad/day (10 Gy/day), which, nevertheless), would be sufficient to cause extensive damage to lymphocytes.

Is this type of estimate more meaningful than the traditional estimate of average radiation dose to an organ? In a recent workshop on lung dosimetry, Fisher expressed his feeling that microdose-response relationships may be difficult to interpret, and his comments concerning microdosimetry are especially pertinent to this discussion (*25*). He stated that "microdosimetry is a special research area designed to provide better understanding of the importance of microscopic patterns of radiation interaction with cells within the broader framework of biochemistry and radiation biology." He further commented that "microdosimetry cannot unravel the complex biological pro-

cesses which follow an irradiation, but it does allow the investigator to take a closer look at radiobiological mechanisms at the cellular level." In his opinion microdosimetry can contribute to investigations by providing the necessary information about the probability of interactions of radiation with sensitive cell types.

Investigators have already considered many aspects of the problems associated with studies utilizing radioactively labeled blood cells. The information presented in this editorial is not new; however, it should serve as a reminder that more studies are needed to determine the limitations necessary for the use of these techniques. As Thakur noted in 1981, the choice of labeling agents will be governed not by small differences in labeling efficiency or ease of preparation but by toxicity considerations and solutions to fundamental problems in cell labeling (*26*).

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