The radioactive labeling of monocytes.

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Chapter 9: SUMMARY, SAMENVATTING.

With the aim of studying a possible relationship between circulating monocytes and Sternberg-Reed cells investigations were started on the specific labeling of monocytes.
In this thesis the literature on the pertinent data has been reviewed and a series of experiments on the monocyte labeling procedure has been described.

In Chapter 1 the objective of these investigations, to relate the origin of the Sternberg-Reed cell in Hodgkin's disease to circulating monocytes and thus to the mononuclear phagocytic system, was stated.

A review of the relevant literature on the characteristics and origin of Sternberg-Reed cells was presented in Chapter 2. It was concluded that no clear answer to the question, from which cell type the Sternberg-Reed cell is originating, can be given by comparing the characteristics of the Sternberg-Reed cell with other cell types because of three reasons:
1. The different techniques used by the various investigators.
2: The small number of patients examined.
3. The malignant nature of the Sternberg-Reed cell.
Therefore another approach, consisting of the administration of labeled cells that will mature into Sternberg-Reed cells was suggested and the requirements for this approach were given.

In Chapter 3, the properties and functions of monocytes in the human body together with the available information on monocyte kinetics were reviewed. The monocyte is active mainly against invading microorganisms by engulfing them directly or after being stimulated to do so by lymphocytes.
Next the problems encountered by measuring accurately the function of monocytes, were discussed. These problems focus on the fact that monocytes do not possess functions that are unique when compared with the other white blood cells. Therefore methods to separate monocytes are necessary. Attention was drawn to the most promising of these methods: counterflow centrifugation elutriation ("elutriation").
Finally monocyte function under pathologic conditions, with special attention to Hodgkin's disease, was reviewed shortly. The general conclusion was that in most diseases the intrinsic monocyte function is normal but that in some

instances (infections, Hodgkin's disease) the monocytes are activated by the conditions caused by the disease.

In Chapter 4 the principles of cell labeling with radioactive compounds were discussed.

These principles can be separated into two parts:

1. Total separation of the particular cell population to be labeled and subsequent labeling with a non-specific radiopharmaceutical.
2. Specific cell labeling in a mixture of cell types based on a well defined affinity of the cell under study for the radiopharmaceutical used.

Next the radionuclides that can be used for cell labeling purposes were discussed with special attention for ^{111}In and its chelates.

The methods that may be used in order to achieve cell labeling by the second principle mentioned above were summarized and the reports concerning monocyte labeling were reviewed. It was concluded that, at present, no reliable method exists for the specific labeling of monocytes.

Finally the principles of radiodosimetry were discussed shortly. This section was focussed on the radiation dose the labeled cells receive because of the intracellular localized radioactivity. The radiation burden is high in comparison to amounts of radiation known to affect cell viability. The method used to calculate the dose from intracellularly radioactivity may give over-estimated values. It was suggested that investigations comparing the biological effects of external- and internal irradiation of cells will have to be performed. The consequences of the radiation dose were discussed and it was concluded that, due to the relative insensitivity of the monocyte, probably no major loss in cell function will occur. Radiation-induced monocyte-related malignancies were considered improbable.

In Chapter 5 a newly developed method for labeling monocytes specifically by phagocytosis of ^{111}In-Fe-colloid without apparent loss of cells was described in detail.

This method consists of several steps:

1. Blood leukocytes are depleted from erythrocytes by accelerated sedimentation of the erythrocytes.
2. A mononuclear cell suspension is prepared by centrifugation on a two-layer human serum albumin (HSA) gradient.
3. After washing the cells, labeling is accomplished by incubating the cells

with ^{111}In-Fe-colloid. The monocytes phagocytosed the colloid particles.
4. The non-phagocytosed colloid is solubilized by ACD and the labeled monocytes are resuspended in autologous plasma.
The cell suspension obtained after centrifugation on HSA was composed of monocytes and lymphocytes. The recovery of monocytes was complete in normals and in patients with newly diagnosed Hodgkin's disease.
Autoradiography of the labeled mononuclear cells showed that:
1. Monocytes are labeled to a significantly higher level than lymphocytes and granulocytes, resulting in a differential labeling of about 90 % in normals and in patients with newly diagnosed Hodgkin's disease.
2. Most of the autoradiographic grains were localized in the cytoplasm of the monocytes.
The viability and sterility of the labeled mononuclear cells proved to be good.
It was concluded that monocytes can be labeled specifically by phagocytosis of ^{111}In-Fe-colloid, with good viability afterwards.

In Chapter 6 some in-vitro functions of labeled monocytes were compared with those of non-labeled monocytes.
No significant differences were found in phagocytosing capacity (measured as chemiluminescence as well as by microscope) using opsonized zymosan particles.
When the (labeled) monocytes were cultured for up to 7 days no effect of labeling on monocyte/macrophage recovery could be found. The amount of cellbound radioactivity decreased rapidly: after 7 days of culture about 20% of the initial amount was cellbound. No re-utilisation of the tracer could be detected.
At least two factors are relevant for this diminishing cell-bound radioactivity:
1. Division of the monocytes during culture, resulting in a 'dilution' of the amount of radioactivity per cell.
2. A "secretion" process that could be partly inhibited by disodium cromoglycate after sevaral days of culture.
It was concluded from these experiments that because of the rapid loss of cellbound radioactivity monocytes labeled with ^{111}In-Fe-colloid cannot be used for long-term kinetic studies in-vivo.

Therefore, the synthesis and labeling of another compound, a cellobiose-

tyramine adduct, that was expected to show a much slower loss from the cell, was performed (Chapter 7). It is known that cellobiose cannot be metabolized by cells, thus, once in the lysosomes, it is rather stable there. In order to label cellobiose with ^{125}I an adduct with tyramine was made and purified. Subsequently the tyramine part of this adduct was radio-iodinated. The synthesis and labeling procedure proved to be rather easy.

Several attempts to label monocytes with this adduct were described:

1. The use of the "piggy-back" system with non-radioactive In-Fe-colloid as the phagocytosed particle. A minor amount of ^{125}I became cellbound with a low differential labeling of the monocytes. It was concluded that this is probably caused by the relatively low phagocytosing capacity of monocytes under the conditions used.
2. The use of ^{125}I-labeled aggregated immunoglobulin. Efforts to label aggregated immunoglobulin with the ^{125}I-tyramine-cellobiose adduct failed to yield a specific activity sufficiently high to perform autoradiography. With aggregated immunoglobulin, labeled with ^{125}I (by the chloramine-T method) to a higher specific activity so that autoradiography could be performed, a very low differential monocyte labeling (less than 30%) was found.
3. The use of liposomes labeled with a lipid-phase marker (^{14}C-cholesteryl oleate). A low uptake of radioactivity by the mononuclear cells was measured, an uptake that was too low to warrant further studies. It was concluded that this low uptake was probably caused by the low phagocytosing capacity of the monocytes under the conditions used.

The conclusion of this chapter was that with the experiments conducted it was impossible to label monocytes specifically with the ^{125}I-tyramine-cellobiose adduct.

Finally, in Chapter 8 three possible directions for future investigations in monocyte labeling were mentioned:

1. Improved cell separation methods in order to produce pure monocyte populations without loss of any specific subpopulation.
2. The use of cell labeling agents that allow labeling in plasma milieu.
3. The use of labeled monoclonal antibodies against monocyte membrane antigens.