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DECAYING RADIOLABELLED LYMPHOCYTES AND METHOD OF USING SAME

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates in general to radiolabelled lymphocytes, and more particularly to T-cell lymphocytes for use in treating or detecting cancer, which T-cells are radiolabelled with an isotope which initially emits β radiation, then subsequently decays to emit another form of radiation.

2. Description of the Related Art

Conventional techniques for treating cancer, such as surgery, radiation, and chemotherapy, cure fewer than half of all cancer patients. In addition, conventional therapy often produces deleterious side effects in the patient, including the production of metastasis, collateral radiation damage and suppression of the immune system.

To address these problems, it has been proposed to increase the cancer cell killing ability of the body's own T-cell lymphocytes. Lymphocytes in general are not able to differentiate a cancer cell from other body cells. However, some cancer cells have a surface chemistry which differentiates them from other body cells. A small portion of the body's T-cells is able to recognize this surface chemistry. As might be expected, the highest concentration of these so-called "selective" T-cells is found at a cancer site. It has been proposed to increase the number of selective T-cells in the body to better attack the cancer. See, Steven Rosenberg, *Scientific American*, May, 1990, p. 62. To do this, a biopsy (cancer nodule) is taken from a known cancer site. The nodule would contain cancer cells, normal body cells, non-selective T-cells and selective T-cells. The

nodule is treated with enzymes to separate the various cells into a single cell suspension. Then, the single cell suspension is chemically treated to cause the T-cells to proliferate. The biomolecule interleukin-2 is often used for this purpose. The single cell suspension is allowed to culture for 30-45 days in the interleukin-2, and the T-cells which are proliferating attack the cancer cells so that a substantial portion of the cancer cells are killed. Then, the T-cells are reinjected into the body. According to the *Rosenberg* method, 200 billion T-cells may be infused into the patient. Interleukin-2 is injected with the T-cells so that the T-cells continue to proliferate.

While the above method is relatively successful, there have been several problems associated therewith. First, a T-cell is only able to kill a cancer cell to which it bonds. Second, because not all cancer cells have a surface chemistry which differentiates them from other body cells, not all cancer cells can be recognized by the selective T-cells. Third, it has been found that the injection of interleukin-2 directly into the body causes harmful side effects, such as an excessive immune response.

Another proposal is to isolate antibodies which selectively bond to cancer cells, introduce radioactive isotopes into the antibodies and reinject the antibodies into the patient. However, because antibodies are relatively small chemical molecules they are not able to carry much radioactivity. Therefore, the number of antibody molecules injected must be very large.

A further proposal is that, once the single cell suspension of proliferated T-cells has been obtained (by the Rosenberg method), β-emitting radioactive isotopes can be introduced into the T-cells. This method is described in U.S. Patent No.

4,935,223 to Phillips, and in U.S. Patent No. 4,707,352 to Stavrianpoulos, both references being hereby incorporated by reference for all purposes. To introduce the radioisotope into the cell, one approach is to synthesize organo-metallic nutrients, such as glucose, or selected amino acids using a β-emitting radioisotope. These nutrients will be ingested by the T-cells in the culture, thus radioactivating the cells. The radioactivated T-cells are then infused into the patient as was done for the non-radioactivated T-cells. The β-emitter is said to be "labeled", because through the selective T-cell, it is directed to a specific portion of the patient (cancer cells). The problem with this method is that the range of a β particle (electron) is several millimeters. Therefore, once the isotope reaches the cancer cell and radiates emitting particles, the β particles harm tissue other than cancerous tissue. Moreover, β emission is not extremely successful in killing body cells. That is, many of the cancer cells survive.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method and product which uses the body's own T-cells to treat cancer in which T-cells are able to kill more than just the cancer cells to which they bond.

It is a further object of the present invention to provide a method and product for treating cancer in which interleukin-2 is not required to be injected directly into the body.

It is yet a further object of the present invention to provide a method and product for treating cancer in which healthy cells surrounding cancerous cells are not substantially harmed.

It is still a further object of the present invention to provide a method and product for treating cancer using radiation

which is more effective at killing cancer cells than radiation used by previous methods.

In accordance with the present invention, radioactively labeled lymphocytes for therapeutic use and a method of forming same are provided. A radioactively labelled T-cell lymphocyte includes a T-cell containing a β-emitting isotope which decays to an α-emitting isotopes. Alternatively, the T-cell may contain a β-emitting isotope which decays to an x-ray or γ-emitting isotope. A therapeutic radioisotope treatment method is provided which includes removing a cancerous nodule containing T-cells from a mammal, introducing into the T-cells a β-emitting isotope which decays to an α-emitting isotope and infusing the T-cells containing the β-emitting isotope which decays to an α-emitting isotope into the mammal. As an alternative to this method, a β-emitting isotope which decays to an x-ray or γ-emitting isotope may be introduced into the T-cell. Then, after the radioactive T-cells are infused into the mammal, the mammal is monitored with a radiation detector to determine the location of the T-cells containing the radioisotopes.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawing illustrates several aspects of the present invention, and together with the detailed description, serve to explain the principles of the present invention. In the drawing:

Fig. 1 is a chart showing the relative survival rates of cells treated with high Linear Energy Transfer (LET) radiation and cells treated with low LET radiation.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

According to the present invention, radioactively labeled T-cell lymphocytes for therapeutic use and a method of forming same are provided. According to the present invention, a T-cell is labeled with a radioisotope which is initially a β-emitter but then decays to an isotope having another type of radioactive emission.

First Embodiment

According to a first embodiment of the present invention, a radioactively labeled T-cell is provided which T-cell contains a β-emitting isotope which decays to an α-emitting isotope. In this manner, it is possible to introduce the T-cell into the mammal's body while it is a low Linear Energy Transfer (LET) radiator (β-emitter), low LET radiation being less harmful to mammals. After the T-cells are injected, the selective T-cells find their way to the tumor cells and bond therewith. As described more fully later, the radioisotope is selected so that it decays to an α-emitter after reaching the cancer site.

The α-emitter (high LET radiation) is very effective in killing cancer cells. Fig. 1 is a graph showing the relative survival rates for cells exposed to low LET radiation and high LET radiation. In Fig. 1, the survival fraction is plotted on a logarithmic scale on the Y-axis and the rads of radiation is plotted on the X-axis. As can be seen from Fig. 1, it takes approximately 900 rads of low LET radiation to kill 99% of the cells exposed thereto (.01 survival fraction). However, it only takes about 200 rads of high LET radiation to kill 99% of the cells exposed thereto. Therefore, α emission (high LET) is much more effective at killing cancer cells.

The range of an α particle is several tens of microns. This is approximately the thickness of one layer of cells. Therefore,

α emission will kill only one layer of cells beyond the cancer cells. This is desirable in that it allows cancer cells without the recognizable surface chemistry to be killed an also allows neighboring cells which may become cancerous to be killed.

According to the method of the present invention, a biopsy is taken to remove a cancerous nodule from a cancer site. At the cancer site, approximately 30% of the T-cells are selective T-cells, whereas in other portions of the body, the concentration of selective T-cells is approximately only 1%. Then, a single cell suspension is formed from the cancerous nodule using enzymes, as was done in the related art. The enzymes may also have a catalatic affect. The single cell suspension is chemically treated to cause the T-cells to multiply and proliferate. The biomolecule interleukin-2 may be used to chemically treat the T-cells. Then, the cancer cells and proliferated T-cells are allowed to incubate until a substantial portion of the cancer cells die naturally and are killed by the T-cells. This period may be between 30 and 45 days. The following Table 1 is a table of the potential radioisotopes which may be used for the first embodiment.

TABLE 1

Radioactive Isotopes

Parent	Half-life	Radiation	Daughter	Half-life	Radiation
Cf 253	17.6days	β-99%	Es 253	20.47days	α-90%
Fm 253	3days	EC-89%, α-11%	Es 253	20.47days	α-90%
Es 254m	39.13hr	β-99%	Fm 254	3.24hr	α-99%
Es 255	38.3days	β-91%, α-8.5%	Fm 255	20.1hr	α-93%
Es 246	7.3min	EC-90%, α-10%	Cf 246	35.7hr	α-99%
Pa 230	17.7days	EC-89.6%, β-10.4%	U 230	20.8d	α-99%
Pa 230	17.7days	EC-89.6%, β-10.4%	Ac 226	29hr	β-80%, EC-20%
		Ac 226 decays to-	Th 226	30.9min	α-98%
		decays to-	Ra 222	38sec	α-96%
		decays to-	Rh 218	.035sec	α-99%
Ra 225	14.8days	β	Ac 225	10.0days	α-92%
Pb 213	10.2min	β	Bi 213	47min	β-97%
		Bi 213 decays to-	Po 213	1sec	α
Pb 212	10.64hr	β	Bi 212	60min	β-64%, α-36%
		Bi 212 decays to-	Po 212	.1sec	α-100%
At 210	8.3hr	Ec-99%	Po 210	138days	α-100%
Bi 210	5.013days	β-99%	Po 210	138days	α-100%

EC = Electron Capture

While Table 1 lists a series of isotopes which may be used, the invention is not limited to isotopes. In fact, most heavy metals will have several isotopes which would be suitable. The first column shows the parent isotope. The second column shows the half- life of the parent isotope. The half-life should be

approximately the same as the time it takes for the injected T-cell to reach and bond with the cancer cell. If the half-life is too short, there may be harmful α emission before the T-cell reaches the cancer site. If the half-life is too long, there will be an undesirable long time until the therapeutic effect is achieved. With this criteria, it is possible to use isotopes other than those appearing in Table 1. In the third column is the type of radiation emitted when the parent decays. It can be seen that of the isotopes shown, the parent cell commonly emits β emissions. In the fourth column is the resulting daughter cell. In the fifth column is the half-life of the daughter cell. It is desirable to choose an isotope having a daughter cell with a very short half-life. In this manner, the α emission will occur shortly after decay to the daughter cell, which decay will preferably occur at approximately the same time the T-cell bonds with the cancer cells. Therefore, the therapeutic α emission will occur shortly after the T-cell bonds with the cancer cell. In Column 6 is the type of radiation emitted by the daughter cell. It can be seen that, of the isotopes listed, the daughter cells predominately emit α radiation.

A large percentage of the radioactive T-cells will not bond with a cancer cell. This is to a large extent due to the fact that many of the radioactive T-cells are not selective. The non-bonding T-cells will be disbursed throughout the body. However, the concentration of radioactive T-cells throughout the body is low enough that substantial damage will not be done to healthy tissue. Moreover, when radiation kills mammal cells, it does so by damaging the spindles. Thus, the mammal cells die during mitotic division. Cancer cells rapidly divide (which is believed to be the result of the oncogene). Therefore, cancer cells are more susceptible to being harmed by radiation. To the contrary,

healthy body cells are not dividing as rapidly as cancer cells, and therefore are as harmed by radiation.

According to the present invention, it is possible to remove the non-bonded radioactive T-cells disbursed throughout the body. This is done by first removing blood and separating T-cells from other blood cells using conventional techniques. Then, the T-cells can be centrifuged. The radioactive T-cells, those with a heavy metal isotope therein, are heavier than the non-radioactive T-cells and therefore separate therefrom. The non-radioactive T-cells can then be infused into the patient.

Second Embodiment

The second embodiment of the present invention is directed to a diagnostic method. According to the second embodiment, a biopsy is performed to remove a cancerous nodule, as was done in the first embodiment. The T-cells in the cancerous nodule are proliferated in a single cell suspension, and the single cell suspension is cultured and introduced with a radioactive isotope in the same manner as in the first embodiment. However, according to the second embodiment, the radioactive isotope is an isotope which is literally a β-emitter which decays to a γ-emitter or an X-ray-emitter.

The parent radioisotopes which are suitable for the second embodiment should have a half-life similar to that of the radioisotopes suitable for the first embodiment. Again, this is so that there would be substantially only β emission until the isotope reaches the cancer site and so that the T-cell is not killed before reaching the cancer site. The following is a table

of isotopes which are suitable for the second embodiment.

Table II

Radioactive Isotopes - Gamma Emitters

Parent	Half-life	Radiation	Daughter	Half-life	Gamma Energy (KeV)
Bk 249	314 days	β-	Cf 249	360 yr	333
Bk 248	23 hr	β-	Cf 248	350 days	18
Cf 245	44 min	EC	Bk 245	5 days	253
Pu 246	10.85 days	β-	Am 246	25 min	107
Pu 234	26 min	EC	Np 234	4 days	109
Th 234	24 days	β-	Pa 234	6.75 hr	100
Rn 211	16 hr	EC	At 211	7 hr	670
Po 206	8.8 days	EC	Bi 206	6 days	184, 343, 516
Bi 204	11.6 hr	EC	Pb 204m	67 min	375
Pb 200	21.5 hr	EC	Tl 200	26 hr	368
Au 193	17.5 hr	EC	Pt 193m	4.3 days	67
Pt 188	10.2 days	EC	Ir 188	41.5 hr	1551
Ir 189	13.3 days	EC	Os 189m	5.7 hr	10.6
W 188	69.4 days	β-	Re 188	16.7 hr	155
Ta 183	5 days	β-	W 183m	5 sec	53
W 178	22 days	EC	Ta 178	9 min	93
Lu 177m	155 days	β-	Hf 177m	1 sec	228
Lu 171	8 days	EC	Yb 171m	8 days	76
Er 172	49.5 hr	β-	Tm 172	63 hr	80

Tm 167	9.6 days	EC	Er 167m	2 sec	208
Tb 153	55 hrs	EC	Gd 153	242 days	99
Nd 140	3.3 days	EC	Pr 140	3.4 min	511
Ce 144	284 days	β-	Pr 144	17 min	695
I 131	8 days	β-	Xe 131m	12 days	164
Zr 95	65 days	β-	Nb 95m	90 hr	235
Zr 88	85 days	EC	Y 88	108 days	898
Rb 83	100 days	EC	Kr 85m	2 hr	9
As 71	62 hr	EC	Ge 71	11.4 days	9
Ni 56	6 days	EC	Co 56	77 days	511, 847
Cr 48	23 hr	EC	V 48	16 days	511, 983

EC = electron capture

The purpose of having the daughter cell emit X-ray or γ radiation is that these types of radiation have a longer range than either β or α radiation. Therefore, with the use of radiation detectors, it will be possible to determine where the radioactive T-cells are located, and hence, where the malignancy has spread. For example, a compton camera may be used to image the daughter isotopes.

Numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that

the invention may be practiced otherwise than as specifically herein.

ABSTRACT OF THE DISCLOSURE

Decaying radiolabelled lymphocytes and a method of using same are disclosed. A radiolabelled T-cell lymphocyte including a T-cell containing a β-emitting isotope which decays to an α-emitting isotope is provided. Alternatively, the T-cell may contain a β-emitting isotope which decays one of an x-ray and γ-emitting isotope. A therapeutic radioisotope treatment method includes the steps of removing a cancerous nodule containing T-cells from a mammal, introducing into the T-cells a β-emitting isotope which decays to an α-emitting isotope and infusing the T-cells containing the β-emitting isotope which decays to an α-emitting isotope into the mammal. As an alternative, the treatment method may introduce into the T-cells a β-emitting isotope which decays to an x-ray or γ-emitting isotope. Then, according to the alternative method, after the radioactive T-cells are infused into the mammal, the patient is monitored with a radiation detector to determine the location of T-cells containing the isotopes.

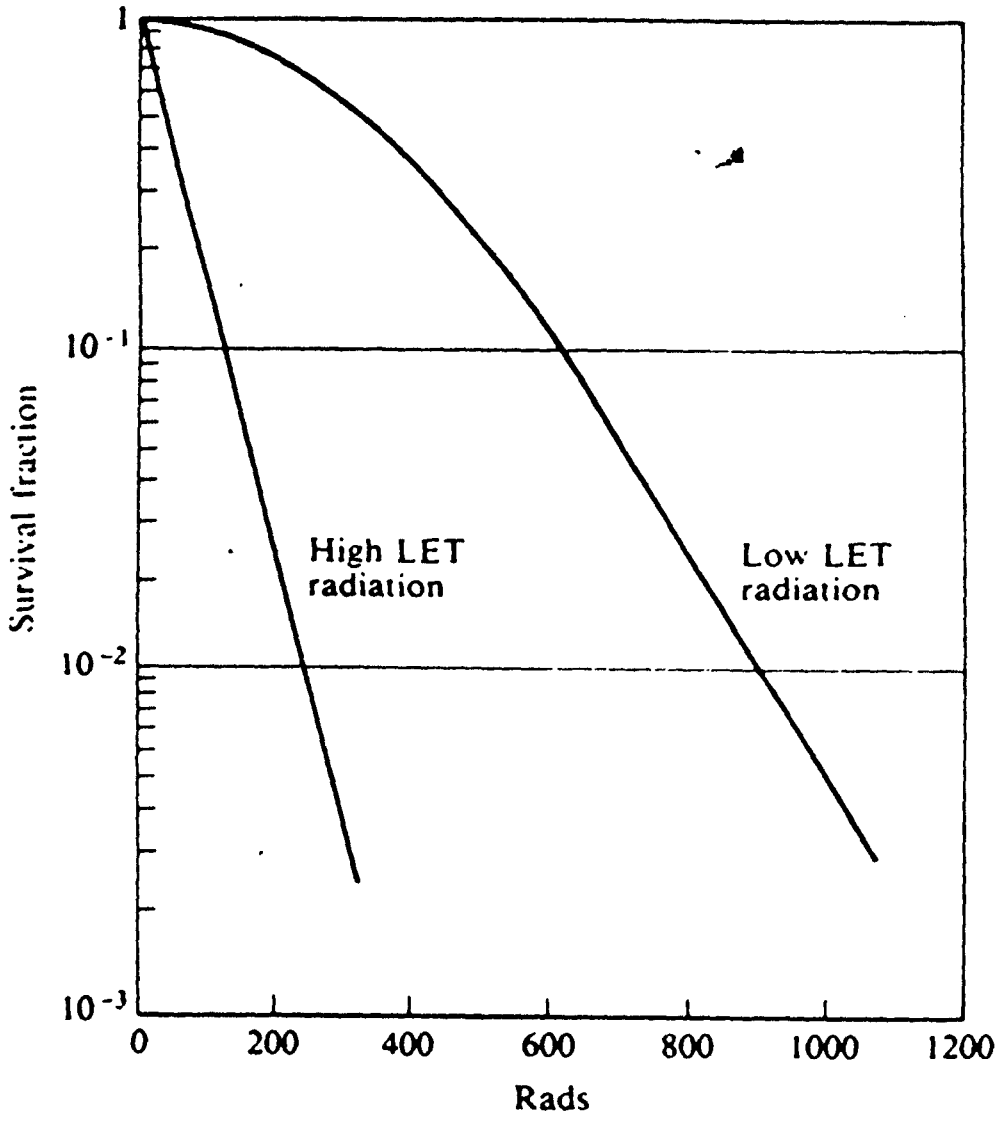

Fig. 9.3 Survival curves for cells exposed to acute doses of radiation.

FIGURE 9.1