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RE: Use of Formalin to Create Cellular Environments for Stem Cell Differentiation

Dear Michael:

Our earlier work, Drake WP et al, “Preservation of Cellular Antigenicity of Tumor Cells by the Use of Formalin Fixation”, Cancer Research 32:1042-1044, 1972, has been considered more significant than earlier thought. This paper has been cited 94 times according to Pub Med (the official online repository of medical reports for the National Library of Medicine), and 105 times by Google Scholar, as the basis for other scientific studies.

Because of the number and breath of citations to our earlier work by others, I wanted to revisit our findings with an eye toward applications in stem cell therapies and the creation of precursor stem cells programmed to selectively differentiate into the desired tissue.

The most recent study citing our work is the Italian group of Pizza G et al “Allogeneic Gene-Modified Tumour Cells in Metastatic Kidney Cancer. Preliminary Report”, Folia Biologica (Praha) 49:147-159 (2003). The authors harvested tumour cells from patients, treated them with formalin, and reinjected the formalin treated cells as a vaccine. They stated that as of that time, the study was the first clinical trial using whole formalin-fixed autologous tumour cells as a vaccine. The authors report a statistical benefit to patient longevity which they attribute to the formalin fixed cells used in the vaccine, and conclude: “...the clinical results reported, the high compliance of the patients to the protocol [i.e. no toxicity], and the low cost of the proposed techniques are encouraging and warrant continuation of this investigation” (insert added). The problem with the study, as noted by the authors, was that they did not use the formalin fixed cells alone as a vaccine, but admixed them with live non-patient allogeneic renal kidney tumour cell line transfected with a gene to produce interleukin-2. However, because the control group of patients were receiving standard IL-2 therapy throughout, the authors appear to tentatively favor the conclusion that
the formalin-fixed cells used in the vaccine led to the heightened immune response measured by mixed lymphocyte reaction, and consequently to increased longevity.

For me, the most interesting comment from that report was that “the activation of cellular immunity requires synergistic signals, including presentation of specific tumour antigens, co-stimulatory signals and the propagation of the immune response via cytokine release...” [emphasis added].

Although we were at the time of our studies interested in whether formalin fixation could preserve antigenic determinants so as to invoke an immune response, the work actually demonstrated that formalin fixation enables the maintenance of cells in a morphologically intact state. We further showed that not only was the cell membrane fixed with respect to antigenic determinants, but also that antibody directed to the cells could be fixed in place by formalinization. Ungaro PC et al, “The Formalinization of Antibody to Tumor Cells in Altering the Immune Response”, Cancer Research 32:2241-2247 (1972).

It is clear that cellular interaction occurs membrane to membrane. The so-called handshake. And at any given time, a cell will be involved in various functions such as producing an enzyme, generating proteins, generating hormones, immunity functions, excretion, repair and so on. A cell suspension would have a population of cells all doing different kinds of things...undertaking differing tasks, and these different tasks would be reflected in the membrane.

The treatment of a cell suspension with formalin would be like a photograph freezing the individual cells’ functions at that moment. If one cell was in the process of releasing a protein, that protein would be fixed to the cell by the formalin. All the things that cells do are reflected at the cell membrane. When we speak of a cellular environment, we mean all the different kinds of membranes and different statuses of the cell membranes at the time. Formalinization does a whole lot more than just result in cells with fixed antigenic determinants...it fixes as well all the other various molecules, messenger proteins, glycoproteins, mRNA and the like related to the cell’s function at that particular time.

The technique of formalin fixation would permit one to create various “cellular environments” one could keep on a shelf to use as needed. A liver cell suspension could be fixed, a kidney cell suspension etc. A patient’s peripheral lymphocytes could be fixed to preserve the antigenic determinants for example.

The use of formalin to create whole cellular environments has at least 3 important applications in stem cell preparations and therapy:

(1) Embryonic Stem Cells exposed to, say, a formalin fixed preparation of a patients cells would be expected to take on and express the patient’s antigenic determinants as their own, REGARDLESS of the source of the stem cells.

(2) Embryonic Stem Cells mixed with a formalin fixed suspension of particular tissue, such as liver cells, would be expected to differentiate into liver cells.

(3) Formalin fixed cells may be able to serve as “feeder cells” in growth media, making it easier to grow and maintain cell lines, and replacing the other types of non-human feeder cell types. Formalin fixed cells may become an essential component of growth media for some types of desired tissue.
While the injection of stem cells into the abdomen has led to tumour formation and/or a multidifferentiated cell mass, the injection of stem cells into a particular tissue has always resulted in differentiation toward that particular tissue. This is because the cellular environment into which the stem cell is placed is the most important determining factor in what that cell will become. There is a constant thread of concern that one sees many times in the literature to the effect that if one were attempting to transplant human embryonic stem cells for therapy and accidentally transplanted some undifferentiated cells, that the undifferentiated cells might result in a tumor. While many scientists have expressed this same concern, I have yet to come across a single paper in which this has actually been observed. To the contrary, many clinical findings coming out of Russia where they are treating many diseases with various stem cell methodologies has shown: (1) no graft vs host rejection; (2) no tumor formations. Moreover, we are all carrying undifferentiated stem cells that find their way into different tissues and differentiate into those tissues in order to participate in repair processes. And this occurs without tumor formation.

My belief is that the cellular environment controls absolutely. That is the whole concept of stem cells: that they are undifferentiated and then are disbursed into various cellular environments wherein they then differentiate into those tissues. A stem cell will only differentiate into a tumor cell if it ends up in or is placed in tumor tissue.

There is only one human genome. Consequently, each individual contains the DNA to create every HLA antigenic pattern. Consequently in my view, the source of human embryonic stem cells is of no matter whatsoever provided there has been no triggering of the construction of the cellular antigenic pattern prior to transplant.

Rather than go about trying to create a “bank” of cell lines that can be matched to any patient similar to blood typing or tissue typing for organ donation, [see e.g. http://www.internationalstemcell.com], it would be better to create methodologies in which the recipient-patients own cells are used to create fully compatible stem cell lines. Patient-specific therapy...that is the beauty of this new field.

I continue to believe that the source of human embryonic stem cells is of no matter in therapy because of the deteminative feature of the end environment. However, the formalin fixation of tissue suspensions may be able to create the in vitro cellular environment necessary to generate a patient specific cell type for transplantation, and this may in the end be eventually accomplished by the simple addition of formalin fixed cells to growth media. I hope in the future that we may be able to initiate some clinical experiments overseas to answer these questions.

Best Regards,

Walter

Walter P. Drake BA,MA,JD

P.S. This note may be cited as: Drake WP, "Use of Formalin to Create Cellular Environments for Stem Cell Differentiation", Letter to Michael R. Mardinaey Jr. M.D., September 23, 2007