

Wilsede Meeting 1988

Keynote:

Alexander J. Friedenstein (Moscow):

On stromal-hematopoietic interrelationships. Maximow's ideas and modern models.

Audio

URL: <http://www.science-connections.com/Wilsede-Activities/multimedia/audio.html#friedenstein>

DOI: 10.3205/wsc-2009-en-000041.01

This **transcript** was prepared by Liudmila Lashkouskaya, René J. Hornung and Claudia Koltzenburg (Cellular Therapy and Transplantation, <http://www.ctt-journal.com>)

URL: [http://www.science-connections.com/books/other/On stromal-hematopoietic interrelationships AJ Friedenstein 1988 Wilsede.pdf](http://www.science-connections.com/books/other/On_stromal-hematopoietic_interrelationships_AJ_Friedenstein_1988_Wilsede.pdf)

DOI: 10.3205/wsc-2009-en-000041.02

License: Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported, <http://creativecommons.org/licenses/by-nc-sa/3.0/>

Full article by the same author, on the same issue, with slides:

Stromal-hematopoietic interrelationships: Maximov's ideas and modern models

A. Friedenstein

Republished from Modern Trends in Human Leukemia VIII (1989), Ed. R. Neth, with kind permission by Springer Science and Business Media

Cellular Therapy and Transplantation (CTT), Vol. 1, No. 3, 2009

doi: 10.3205/ctt-2009-en-000033.01

<http://www.ctt-journal.com/1-3-en-friedenstein-1989.html>

[Introduction by Malcolm A.S. Moore]

Malcolm A.S. Moore:

Doctor Friedenstein is a person that I think laid the foundations for the substantial areas of research that was carried out subsequently by other people, including Mike Dexter and myself. We owe him a major debt for his pioneering work and it is particularly exciting for us to have him come to this meeting. It is one of... I think, talking to him, he said the second time that he has been outside the USSR at a scientific meeting.

So we'll begin with the first talk: "On stromal-hematopoietic interrelationships. Maximow's ideas and modern models." - Doctor Friedenstein?

Alexander Friedenstein:

It's certainly an honour to speak here about Maximow's works in connection with modern problems of hemopoietic micro-environment. It's also a great pleasure for me to participate in this meeting. So I want to thank Doctor Neth for giving me the possibility to come to this meeting.

The idea of stromal-hemopoietic interactions was part of Maximow's theory of hemopoiesis, which he proposed more than 60 years ago. Maximow assumed that differentiation of hemopoietical stem cell descendants depends on what he called "local conditions", which are generated from bone marrow stroma. Maximow's theory was far ahead of his time. And though Maximow was highly respected in the scientific community, his concept of local stromal conditions was met with skepticism. But now, this Maximow's idea raises no doubt. It was directly proved by Dexter cultures and in fact constitutes an essence of problem of the hemopoietic micro-environment as it is perceived in modern hematology.

But the main question, that is which of the stromal cells establish the micro-environment, continues to provoke discussions. According to Maximow, the cells in question are the bone marrow fibroblasts or reticular cells.

I'm going to speak about their micro-environmental functions and about possible diversity in hierarchy of their clonogenic precursors.

At present, the micro-environmental functions of stromal cells can be analyzed using two different procedures. The first way is to test the ability to transfer the micro-environment in vivo. The second way to test the capacity of maintaining hemopoiesis in vitro and to produce hemopoietic growth factors.

The first model... First slide, please.

The first model is based on the results of heterotopic transplantation, either of marrow fragments or of marrow cells itself, which are grafted under, for example, renal capsule. And these grafting results in the formation of new ossicles with the medullary cavity in which the repopulated recipient cells proliferate and differentiate as in the ordinary bone marrow. In the heterotopic bone marrow organs, all the hemopoietic cells, or most of them, are of the recipient origin, which means that they are the cells, which repopulated the new micro-environment. At the same time, there are direct proofs, that the heterotopic micro-environment is established by engraftment of donor cells, which remain non-replaced by the recipient cells.

Thus, heterotopic transplantation makes it possible to test whether the marrow fibroblasts can transfer and establish the micro-environment. This was done with deployed cultures of marrow fibroblasts. We grafted them under the renal capsule.

And on the next slide you'll see that... the result was the formation of new bone marrow organs with the medullary cavity filled with hemopoietic cells. And the hemopoiesis, which took place in this medullary cavity was also the usual one including proliferation of stem cells, formation of committed precursors, and their differentiation.

Doctor Patt has also shown, that dependent of the origin of the engraftment marrow fibroblasts, they transfer not only the general pattern of the micro-environment, but also its details, for example the density of hemopoietic cells in the would-be hemopoietic organs or whether it would be a red or yellow marrow.

So, cultured marrow fibroblasts can transfer and establish the bone marrow micro-environment in vivo. On the other hand, cultures of pure marrow fibroblasts, as it was shown by Brockbank, may also be used successfully as underlayers for Dexter culture. They also produce, as it is well known, a vast set of hemopoietic growth factors.

Thus, marrow fibroblasts are the micro-environmentally competent cells. But their population, most probably, is a heterogeneous one. And the next step in studying the hemopoietic micro-environment is to find out the genetic diversity of different types of marrow fibroblasts. At present, all fibroblasts, including those from hemopoietic and non-hemopoietic organs, look quite alike.

Next slide, please.

There are typical marrow fibroblasts in culture and as you see, they can't be distinguished from other fibroblasts, say from skin and so on. And there are no cyto-chemical markers and differences to distinguish fibroblasts of different origin.

2nd slide, please.

All of them synthesize in cultures: one- and three-type collagen. It is the main mark of fibroblasts in cultures. They lack the markers of macrophages and most markers of endothelial cells.

However, the diversity of fibroblasts of marrow origin and the diversity of... between fibroblasts of marrow origin compared to, say, stromal... spleen fibroblasts certainly exists. For example, if rabbits' spleen fibroblasts are engrafted under the renal capsule, they form not a bone marrow or an organ but some sort of a lymphoid organ where no hemopoiesis takes place and, on the contrary, which is filled with the proliferating lymphocytes and no bone capsule is found in such a marker.

The source for marrow fibroblasts cultures are stromal colony forming cells or stromal colony forming units, CFU-f.

Second slide, please. Not so quick. Well...

The colony-forming units of stromal fibrocytes or stromal fibroblasts are tested in such a way, that single marrow suspensions are expanded at low initial density in liquid marrow cultures. The precursors of fibroblasts, CFU-f, are highly adherent cells so they stick to the surface of the blood vessels. And in ten days they form fibroblasts colonies, which are cell clones. This fibroblasts may be passaged in two different ways. First you can passage the total amount of colonies or you can passage the colonies one by one. In the first way you get the multi-colony derived fibroblasts strains because they are deployed and have a high proliferated capacity. At the second way you have the single colony derived fibroblasts strains which are the clone cultures. Then the cultures may be either engrafted heterotopically to learn whether they transfer, or don't, the micro-environment or or they can be transplanted in diffusion chambers to learn the differentiations capacity of the whole of colonies or of separate colonies. It can be easily shown, that micrological CFU-f colonies are certainly heterogeneous.

Second slide... ah, next slide, please.

That is... that are individual colonies stained for alkaline phosphatase. You can see, that some of the colonies are negative, the others are positive.

Next slide, please.

These are from different cultures. Different cyto-chemical reactions can be used and diversity also exists while using this reaction.

Next slide, please.

Some of the colonies consist of spreaded fibroblasts like here, the others of blanket cells.

Next slide, please.

Other colonies are the mixture of two kinds of fibroblasts, or some colonies may be even mineralized. However all this phenotype differences can not serve as good markers of CFU-f diversity because they are non-stable during recloning.

What I know is that CFU-f differ by their differentiation potential. When single colony derived cultures of marrow fibroblasts from the red were tested by transplantation in diffusion chambers it was found, that some of the colonies, some of the single colony derived strands, form simultaneously bone and cartilage.

Next slide, please.

As it is shown here, is the result of transplantation in the chamber of single colony derived fibroblasts which form simultaneously cartilage and bone, two different things.

Some of the single colony derived cultures form only bone or a tissue which resembles the reticular tissue, that one can not be sufficiently sure that it is real reticular tissue. In the chambers, which were grafted with cultures, which form bone and cartilage, some of this reticular like tissue is also formed.

Next slide, please.

Here is this reticular tissue in question.

These fibroblasts... cultures, these single colony derives fibroblasts cultures differ in their capacity to proliferate. Those, who are osteogenic and form simultaneously bone and cartilage have a high proliferated capacity, which provides for non less than 20 cell doublings.

Next slide, please.

CFU-f which initiate the cultures with osteochondrogenic potencies are supposed to be skeletogenic or osteogenic stem cells. Anyway they are highly proliferated common precursors for both osteogenic cells and fibroblasts. The other strains of fibroblasts form only bone tissue and the less reticular like tissue. The distributions of the CFU-f are as it is shown here.

The most interesting CFU-f are those, which give arise to cultures, which form bone, cartilage, and may be reticular tissue. Because only this single cells derived cultures, when grafted heterotopically, form bone organs, which are filled with hemopoietic tissue. This means, that they transfer the hematopoietic micro-environment.

Thus Maximow's hypotheses, that marrow fibroblasts are responsible for bone marrow micro-environment seems to be confirmed by using these experimental models.

One can be speculated, that several types of marrow fibroblasts are participating in establishing hemopoietic micro-environments and the micro-environment is a joint product of a network of different reticular cell types.

Next slide.

In situ the CFU-f...ah... Well, that is the bone marrow organ or the bone marrow cavity in which hematopoiesis takes place, which is formed by grafting of a single colony-derived fibroblast strain.

Next slide, please.

In situ, the CFC-f are outside the cycle. They are arrested in G0 and for a long time it remained unnoticed, that the recruitment into the cycle required stimulation by some hemopoietic cells. It came to light only when the CFU-f assay was performed in marrow cultures from which the non-adherent marrow cells were removed. The results of such an experiment are shown here.

In all the cultures, the marrow cells were planted with low initial density. After one hour the non-adherent cells were removed and the cultures were cultivated for ten days. Now, some of the cultures were supplemented with different numbers of irradiated marrow cells instead of the cells which were removed after one hour of cultivation.

Other cultures were not supplemented with marrow feeder cells. You see, that the colony formation in the not supplemented cultures didn't take place. It means, that CFU-f did not proliferate. The cultures were maintained with different concentration of serum: one percent, twelve percent, and twenty percent. And even with twenty percent of serum no colony formation without supplementary feeder cells took place. But if the cultures were supplemented with feeder cells, than the usual colony formation... the usual efficient colony formation could be obtained and it was the high concentration plus feeder-cells were used.

Next slide, please.

You can see here such cultures. This is the not supplemented with feeder cells colony. And this was the supplemented one. This is the number of planted cells and this is the same family. Now, what was found, that the supplementing feeder cells, which stimulate the fibroblasts colony formation were simply platelets. Now, I must mention, that this cultures were maintained with high serum concentration in the medium. That means that they were cultured with high amounts of PDGF. On the other hand, if PDGF was added into the culture additionally, instead of platelets, there was no effect. Which means, that probably platelets have some colony stimulating factor, which stimulates proliferation of fibroblasts, which are initially in G0 period, which is necessary for CFU-f colony formation.

Thus the CFU-f colony formation depends on stimulating factors, or one of them, which remain to be identified and which is produced by hemopoietic cells or their descendants. That means, that the interaction of hemopoiesis and stromal cells maybe goes in two directions. This stimulation of hemopoietic cells, which is usually meant when we are speaking about the micro-environment, but it takes place also in the opposite direction. The hemopoietic cells can stimulate and maybe are the necessary stimulators of proliferation of the resting stromal marrow cells.

Now to finish my talk I would like to return to Maximow's last work, which appeared in 1928 in "Archiv für experimentelle Zellforschung". It was entitled "Cultures of blood leukocytes: From lymphocytes and monocytes to connective tissue". This work described the development of fibroblasts in plasma-clot cultures of blood cells. However, it was later disproved on the grounds of two objections. Objection one was that fibroblasts could be components of the vessel walls which contaminate blood during sampling. Objection two was that they were not true fibroblasts.

The problem of circulation of fibroblasts precursors in blood sometimes is discussed at present. Objection number one, that means, that the colony of fibroblasts contaminate blood during sampling, turned out to be invalid when CFU-f colony assay was used for culturing blood-circulating blood cells.

Luria from the Gamelaya Institute in Moscow showed, that numbers of CFU-f colonies in cultures or blood cells were in the linear relation to the numbers of the explanted leukocytes and independent from the number of functions, which was made for blood collection.

Now, I wish to demonstrate that the cells in Maximow's blood cultures were true fibroblasts, which means, that they synthesize one and three type collagens.

Next slide, please.

Here are such cultures. You see the individual colonies.

Next slide, please.

And here are the cells, which compose this colonies. They are typical fibroblasts by morphology.

Next slide, please.

This is the other colony, which you see, consists of the same cells of different shapes, but it means nothing.

Next slide, please.

What means is that the cells...the fibroblasts synthesize first type collagen.

Next slide, please.

And third type.

Next slide, please.

Well, before showing you Maximow. I must conclude, that it remains not known, where from CFU-f migrate into blood and were they settle. But their circulation may happen to be significant for understanding of the role of fibroblasts in establishing hemopoietic micro-environment. Anyway the story about circulating CFU-f demonstrates, that Maximow's experimental results, not only his ideas, are so topic that, virtually, Maximow still remains the participant of the present day research. That is why I would like to show you Maximow at work in St. Petersburg.

Maximow on the left.

Next slide, please.

Maximow on the right: he is taking blood from the rabbit.

Next slide, please.

So he is going to centrifuge this blood.

Next slide, please.

Here is the beginning of preparing the cultures in plasma-clot system.

Next slide, please.

Next slide, please.

That's the modern thermostat.

Next slide, please.

That's all. Thank you.

(Applause)

Malcolm A.S. Moore:

Well, it is really nice to see the father of a field... Two fathers actually – this is another one. Questions? We have a time for a couple of questions.

Q: In the single colony transfer experiment, where you had 50% of the colonies being shown for competence for osteogenesis, chondrogenesis in the reticular tissue. Did you do any experiments which tested whether the cells gave rise to this colonies were able to self-renew and make additional colonies? I mean, did they full range of differentiation corresponded anyway with self-renewal?

A. Friedenstein: Do you mean the recloning of cells from initial fibroblasts colonies?

Q: Yes. When you did a single colony transplantation experiment some of the colonies were able to differentiate in to bone cartilage in reticular tissue. Did you do any experiments, for example. to split colonies and transplant some of the cells and replat some of the cells, to try to correlate self-renewal of the clonogenic cells with differentiation?

A. Friedenstein: That is what we did. We had cultures, which were single colony derived.

Q: So, all the colonies that gave rise to bone cartilage in reticular tissue, they're all self-renewed? ... Maybe I talk to you about it after. Maybe it is a bit complex.

Michael A.S. Moore: I think he is trying to establish whether there is the question of pluripotentiality, whether the fibroblasts clone can reclone as a pluripotential cell, capable of giving rise to all the different components of the bone, cartilage, etc.

A. Friedenstein: Yes.

Q: They do? All these colonies do?

A. Friedenstein.: Yes. It's... Well, what we did is that... Maintaining such single cell colonies, single cell cultures, we were grafting them into diffusion chamber on different passages. That means that the line of the common precursor certainly exists at the strain. But what we don't know whether all the CFU-f, which were generated in such cultures, were also polypotential.

Q: Ok.

M. Moore:

Any further question? I would like to mention, that we done some studies with xenografting of human fibroblasts colonies in nude mice and we saw the same pattern of bone development with an interesting chimaeric situation, where some of the murine components are involved in the development of bone, e.g. osteoclast and hemopoietic elements with the other components of the bone being of human origin.

(End of the audio)

License: Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported,
<http://creativecommons.org/licenses/by-nc-sa/3.0/>