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The quest for hematopoietic stem cells in the embryo
- an interview with Françoise Dieterlen-Lièvre

THIERRY JAFFREDO*,[1] and CHARLES DURAND[2]

[1]CNRS UMR7622 and [2]UPMC UMR7622, Laboratoire de Biologie du Développement, Paris, France

ABSTRACT Françoise Dieterlen-Lièvre is probably the scientist who has most contributed to our basic knowledge of developmental hematopoiesis. She has dedicated her career to answering cutting edge questions on the origin of hematopoietic stem cells in the embryo. Her seminal contributions, widely recognized by the scientific community, have paved the way for generations of developmental hematologists questioning the origins of hematopoietic stem cells. After having demonstrated the intra-embryonic origin of hematopoietic stem cells, established the dual origin of the endothelial network in the embryo and revealed the hematopoietic function of the allantois in birds, she has switched to mammals and contributed to demonstrating that the aorta and allantois/placenta are new sites of hematopoietic production in the mouse embryo. The manifold insights generated by the pivotal work of Françoise Dieterlen-Lièvre have created multiple paradigm shifts which continue to challenge the field of developmental hematopoiesis.

KEY WORDS: *embryo,chick, quail, mouse, aorta, allantois, yolk sac, placenta, hematopoiesis*

Introduction

Dr. Françoise Dieterlen-Lièvre has dedicated her career to experimental avian and mouse embryology. From the sixties to the mid seventies, she studied in depth the formation of the pancreas in birds and the emergence of Langherans islets, the subject of her PhD thesis. Soon after the discovery of the quail-chicken system by Pr. Nicole le Douarin, she became interested in Developmental Hematopoiesis and demonstrated in 1975, using the avian model, the existence of an intra-embryonic source for Hematopoietic Stem Cells (HSC). Pursuing the quest for HSC, she demonstrated in 1986 with her team that the aorta was able to produce multipotent hematopoietic progenitors in culture, long before similar questions were asked for the mouse embryo, thus demonstrating the great possibilities offered by the use of the avian embryo. At the same time, she also became interested in the origin of the vascular system in the avian embryo and developed several tools to follow endothelial precursors in the embryo. This interest lasted until the end of the nineties, bringing several major contributions, one of the most well known being the existence of two different endothelial lineages in the embryo, one being associated with hematopoiesis. From the beginning of the nineties, she turned her attention to the mouse embryo and the origin of HSC in this species. In 1993, she unravelled an unexpected hematopoietic activity in the aortic region of the early mouse embryo. In the next few years, she published several seminal papers on the mouse aorta and HSC production. At the same time, she demonstrated, using the avian model, the production of hematopoietic cells by endothelial cells. Last but not least, from 1998, she unravelled the existence of a new hematopoietic site and organ i.e. the allantois. First investigated in the avian model, the question of the mouse allantois rapidly came under scrutiny and she demonstrated that the mouse allantois was capable of autonomously producing multipotent progenitors and that the placenta was an organ where HSC expanded. Françoise Dieterlen-Lièvre was co-director of the Nogent Institute from 1981 to 2000 (see Le Douarin, 2005) . She retired in 2003 but continues her research.

First of all, could you tell us when your interest in developmental biology arose and why you decided to study the embryo?

My interest in biology in general, and more specifically in embryology, was largely incidental. When I was 16, I passed the

Abbreviations used in this paper: AGM, aorta-gonad-mesonephros; HSC, hematopoietic stem cell; P-Sp; para-aortic splanchnopleura.

***Address correspondence to:** Thierry Jaffredo. CNRS UMR7622, Laboratoire de Biologie du Développement, Bat C, 6ème étage; Case 24; 75252 Paris Cedex 05; France. Fax: +33-14-427-3497. e-mail: thierry.jaffredo@upmc.fr

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baccalauréat, which in France opens the door to University, and had no precise calling. My parents were both physicians, but did not particularly encourage their progeny to adopt this track. I suppose studying biology was a close make-believe. Then when I finished my undergraduate studies, I went to see Pr. Etienne Wolff, originator of the French school of avian embryology, who was an acquaintance. He was moving from Strasbourg and creating a laboratory in the *College de France* in Paris, where he had recently been elected. He accepted me as a junior investigator in the new team he was creating. This turn of events was determinant, as developing embryos are fascinating and the experimental possibilities provided by the avian model turned me into an enthusiast.

How did you come up with the idea that the embryo harboured an intra-embryonic source of HSC? At that time, Moore and Owen's hypothesis of the yolk sac origin of HSC was prevalent, if not actually a dogma.

My interest in hematopoietic development arose as a follow-up of my PhD work on pancreatic development. This was in the early sixties, that is, the pre-molecular era of developmental biology. Many scientists were trying to understand the mechanisms of epithelio-mesenchymal interactions. E. Wolff had designed a culture system that allowed organ rudiments to develop *ex vivo*, while preserving their three-dimensional structure (Wolff and Haffen, 1952a). In the laboratory, each person worked on a different rudiment. Seminal contributions were made at that time, for instance by Katty Haffen about gonadal differenciation (Wolff and Haffen, 1951, 1952b), by Philippe Sengel about skin development (Sengel, 1955 a,b), by Nicole Le Douarin, who discovered at that time the sequential inductions required for liver development (Le Douarin, 1964a; Le Douarin, 1964b; see interview with Nicole Le Douarin in this issue: Durand and Jaffredo, 2010).

Against this background, I was assigned the pancreas. I spent a lot of time establishing the ratio between endocrine and exocrine pancreas (the so-called 'Richardson and Young ratio') and found that the islets of Langerhans are prevalent in early development and synthesize glucagon and insulin long before the exocrine tissue produces enzymes (Dieterlen-Lièvre, 1970 a,b).

During ontogeny, the spleen rudiment is a mesodermal cap continuous with pancreatic mesoderm. As several hematopoietic organ rudiments are built from endoderm and mesoderm, for instance the thymus, I wondered whether the pancreatic endoderm had a role in the formation of the spleen. This was the starting point of my interest in blood-forming organs. At that time (1972), Claude Martin, who was a micro-surgery champion, invented sophisticated new techniques, including the 'yolk sac chimera'. She published the technique (Martin, 1972), but she was involved in analyzing the developmental cascade of the three embryonic kidneys with Yvon Croisille and Madeline Pinot, so that she did not exploit these chimeras.

Could you explain what these chimeras are?

First a point of history: it was in 1969 that Nicole Le Douarin imagined transplanting quail rudiments into chick embryos, an idea which was based on her noticing a marked difference between the cell nuclei of the two species (see Coutinho, 2005). She thus devised the labelling method which allowed her to unravel many developmental processes, relating notably to the nervous and the immune systems (Le Douarin, 1969). The 'yolk sac chimeras' devised by Claude are built surgically by grafting the central area (the future body) of a quail blastoderm onto the peripheral area of a chick yolk embryo (the future yolk sac). This is done in the egg at two days of incubation and the operated embryo can successfully pursue its development in about ten per cent of the cases. These heterospecific chimeras are raised until day 13 of incubation; later the yolk sac, disproportionate in relationship to the embryo, fails to incorporate into the body wall (Dieterlen-Lièvre, 1975). A couple of years later, homospecific chimeras built between two strains of chicken were raised successfully to adulthood (Lassila *et al*., 1978).

When I began speculating about the hematopoietic system, this novel type of chimera was an obvious tool. At that time, I had no preconceived idea; it was more or less unstated in my mind that this approach would validate Moore and Owen's experiments. Their interpretation, according to which all Hematopoietic Stem Cells (HSCs) were born in the yolk sac (Moore & Owen, 1965; Moore & Owen, 1967), had immediately become a dogma in the scientific community.

In a first set of experiments I produced about 10 chimeras which gave very homogeneous results: the thymus, bursa of Fabricius and spleen were all populated by quail cells, which hence did not originate from the yolk sac. I was thrilled, so I sent the paper to *Nature*. The reply received within a week was the following: *"Moore and Owen have already proved the contrary, it is preposterous to return back to this".* I decided to try the *Journal of Embryology and Experimental Morphology* (now *Development*), where it was readily accepted (Dieterlen-Lièvre, 1975). This 1975 paper has recently been elected as the first of a series of 'JEEM Classics' (Dzierzak & Medvinsky, 2008).

In 1978, you published a *Nature* paper on the origin of lymphoid stem cells analysed by means of a chicken yolk sac/chicken embryo chimera. How was the paper received and does it mean that the intra-embryonic source of HSC was accepted by the scientific community?

This piece of work resulted from a collaboration with the finnish team of Paavo Toivanen (Lassila *et al*., 1978, 1982). In these chimeras, the markers distinguishing the two chicken embryos composing the chimera were either different immunoglobulin allotypes or different Major Histocompatibility Complex haplotypes. This work confirmed and extended the conclusions drawn from the analysis of heterospecific chimeras, as the chicken-chicken chimeras hatched and were raised to adulthood. In this make up, where no differences in size and developmental rhythm, nor immune acute rejection could interfere, red cells of yolk sac origin disappeared before hatching and the cells identified through the two markers were all of embryo origin.

The turn of opinions was driven by the acceptance of this paper in *Nature* and, very soon afterwards, of another in Blood (1979). The latter, carried out with Claude Martin and Denise Beaupain, described the replacement of circulating chicken red cells (derived from yolk sac HSCs) by quail red cells (derived from embryonic body HSCs) (Beaupain *et al*., 1979). Amazingly, for ten years, many papers in the field of immunology stated that *'HSCs come from the yolk sac, even though we know it is different in the avian embryo'*. The avian paradigm however had soon been extended to amphibians.

Your demonstration in a 1993 *Nature* paper that the aortic region of the early mouse embryo harbours HSCs has certainly changed the common view?

Nearly twenty years elapsed however before experiments carried out in mice were published back to back in this issue of *Nature*, that you mention, by Alexander Medvinsky, Nina Samoylina, Albrecht Müller and Elaine Dzierzak on the one hand (Medvinsky *et al.*, 1993) and by ourselves on the other (Godin *et al.*, 1993). It is clear that these papers became acceptable because of their identical conclusion, and also because the way of thinking had been modified by the concurring data obtained in other classes of vertebrates. The article by Medvinsky *et al.* examined CFU-S (Colony Forming Unit in the Spleen) activity (i.e short term hematopoietic progenitors) in the tissues from the trunk region of the embryo corresponding to the aorta plus the gonads and the mesonephros. The authors called these tissues 'AGM', a term which became adopted, even though it has no anatomical or embryological meaning, but denotes the impossibility of dissecting these three organs from one another in the very early embryo. We know now that the endothelium of the aorta is responsible for emitting the intra-embryonic HSCs, and I feel strongly that the term 'AGM' should be replaced by 'aorta'. Cells capable of giving rise to colonies in the spleen of irradiated mice were found in this region at days 10-11 of gestation. Our own work, whose conception and methods were proposed and driven by Miguel Marcos and carried out with Isabelle Godin and Juan Garcia-Porrero, bore on earlier tissues (days 8.5-9) in the same region. They comprised the hindgut, the paired rudiments of the dorsal aorta, the vitelline arteries and the mesoderm surrounding these rudiments. We called these tissues 'Para-aortic Splanchnopleura' (P-Sp), the splanchnopleura being the association of mesoderm and endoderm, which is going to give rise to visceral organs. These tissues were tested for their capacity to restore B lymphocytes in immunodeficient (SCID) mice. Interestingly a particular subpopulation, the CD5+ B cells, which reside in the peritoneum, and are not reconstituted by adult bone marrow in irradiated adults, were restored by grafts of P-Sp under the kidney capsule of the SCID adults.

Fig. 1. Françoise Dieterlen-Lièvre at a party at Nogent Institute in 1980.

One of your most relevant discoveries may be the role of the allantois/placenta in the generation of HSC. You have opened an unexplored field that has now major implications for basic biology but also for regenerative medicine. Could you explain how you came up with this idea?

First, I would like to remark on your comment about implications for regenerative medicine. I do not think that pinning down the hematopoietic potential of the placenta will revolutionize the state of the art (see Dieterlen-Lèvre *et al.,* 2010). Cord blood stem cells, discovered and promoted by Elaine Gluckmann in Paris, are now major actors in regenerative medicine. As they are relatively easy to obtain, I do not think they will be superseded by cells from the placenta. But they probably are the product of placental activity, an important point to establish for the sake of knowledge. In that area again, the avian model gave the lead. In the 1979 *Blood* paper (Beaupain *et al.*, 1979), a picture of the sectioned allantois of a quail/chicken chimera clearly showed a blood island-like structure, which encased quail blood-like cells. I decided to pursue this lead, when cultures of whole chicken allantoises in liquid medium gave rise to clouds of red cells. Our classical *modus operandi* was repeated, namely unvascularized allantois rudiments from the quail embryo were grafted (ectopically) into non-compromised chicken hosts, with the result that the host bone marrow was heavily populated by quail HSCs and endothelial cells, a story that you, Thierry, know well, since this was your entry into the world of hemangioblasts. It is fair to mention here that the seminal experiments in my group about the relationship between the 'hemogenic' endothelium of the aorta and HSCs were carried out by Luc Pardanaud. Soon we addressed the issue successfully in the mouse embryo with Marcio Alvarez Silva and Josselyne Salaün (Alvarez-Silva *et al.*, 2003), continuing into the matter with Hanna Mikkola and Stu Orkin (Developmental Cell, 2005) and Catherine Corbel and Josselyne Salaün for the allantois (Corbel *et al.*, 2008). Several groups entered the field along the process, but this is not the place for an exhaustive review!

I know from our frequent discussions that, despite the fact that you spent more than half of a century working on developmental hematopoiesis, you are still fascinated by this field and have revolutionary ideas to explore hematopoietic production in the embryo. What are according to you the

most relevant and still unanswered questions regarding hematopoietic development?

The hemogenic capacity of the endothelium is the next challenge. Is this striking potential restricted to the embryonic aorta, where it is very visible and was experimentally pinned down or is it a generalized potential? If it is, the role of the niche (formerly the microenvironment) must be critical in allowing its expression.

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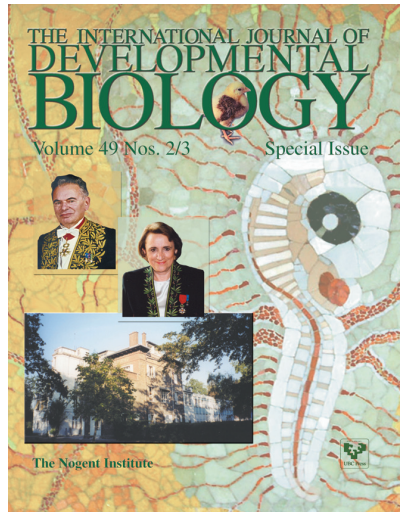

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