Twenty years of research on primordial germ cells

MASSIMO DE FELICI*
Department of Public Health and Cell Biology, Section of Histology and Embryology University of Rome “Tor Vergata”, Rome, Italy

ABSTRACT Just twenty years ago I was preparing a research project centred on establishing methods for the isolation and culture of mouse primordial germ cells (PGCs). The project had been suggested to me by Anne McLaren and was to be developed at the Medical Research Council (MRC) "Mammalian Development Unit" in London under the direction of Anne herself. At that time I was a young postdoctoral researcher at the Institute of Histology and Embryology of the University of Rome “La Sapienza” and did not imagine that my decision to be involved in this project would signal a profound switch in my scientific life. From then on my research would mostly concentrate on primordial germ cell biology. I feel like saying that the modern history of mammalian primordial germ cells began twenty years ago at the MRC Mammalian Development Unit under Anne McLaren’s impulse. It is not surprising that among the most active researchers in the last twenty years in studying mammalian primordial germ cells, three, namely Chris Wylie, Peter Donovan and myself, began their studies under Anne McLaren’s guidance. Over the years, Anne’s suggestions and encouragement were always precious for my studies and her presence marked my most important findings on PGC biology. She often invited me to present the results obtained in my laboratory to workshops and congresses. In the present article some of these results particularly influenced by Anne’s teaching and suggestions will be briefly reviewed.

KEY WORDS: primordial germ cell, gametogenesis, stem cell factor, leukemia inhibitory factor, meiosis.

The long saga of purification and culture of mouse primordial germ cells

Primordial germ cells (PGCs) are the founders of the gametes. They arise at the earliest stages of embryonic development and migrate to the gonadal ridges, where they differentiate into oogonia/oocytes in the ovary and spermatogonia in the testis. When I first became interested in mammalian PGCs, mainly morphological studies describing the main stages of PGC development had been performed. Thanks to their high alkaline phosphatase activity, PGCs had been identified in an extraembryonic region near the yolk sac, early in embryogenesis, and traced in their migratory route towards the gonadal ridges (for a review, see Buher, 1997). In the mouse, PGCs first appear in the early 7-day embryo as a cluster of 50-100 alkaline phosphatase positive cells at the base of the allantois (Ginsburg et al., 1990). From this region, PGCs translocate into the endoderm of the yolk sac and hind gut. At 10 days post coitum, they begin to move by active migration up the dorsal mesentery and into the gonadal ridges where, around 12.5 days post coitum, they differentiate into oogonia/oocytes and spermatogonia in the ovary and testis, respectively (for a review, see De Felici, 2000).

The main goals of my early experiments carried out in Anne McLaren’s laboratory at the MRC Mammalian Development Unit was to develop methods for the isolation, purification and culture of mouse PGCs at different developmental stages. We anticipated that the possibility of studying PGC behaviour in vitro would allow uncovering some of the mechanisms underlying their development. Using a relatively simple method based on EDTA and mechanical disaggregation of gonadal tissues, we obtained an enriched PGC population from post-migratory stages (12.5-13.5 days post coitum embryos). Moreover, the use of a discontinuous Percoll gradient allowed us to increase the purity of such PGC populations up to 80-90% (De Felici and McLaren, 1982). Their very low numbers, their migration throughout different tissues and the lack of specific antibodies, rendered difficult the task of isolating PGCs during their migratory stages. Nearly thirteen years later in my laboratory in Rome, the availability of monoclonal antibodies able to bind to PGC surface molecules (SSEA-1, Fox et al., 1981; EMA-1, Hahnel and Eddy, 1986; TG-1, Donovan et al., 1986) and the introduction of magnetic cell sorting techniques, allowed us to develop efficient methods for purifying PGCs from both migratory (10.5-11.5 days post coitum) and post-migratory (12.5-13.5 days post coitum) embryonic stages (De Felici and Pesce, 1995; Pesce and De Felici, 1995) (Table 1). Afterwards

Abbreviations used in this paper: EG cells, embryonic germ cells; ES cells, embryonic stem cells; LIF, leukemia inhibitory factor; PGCs, primordial germ cells; SCF, stem cell factor.

*Address correspondence to: Massimo De Felici. Dipartimento di Sanità Pubblica, Università di Roma “Tor Vergata”, Via di Tor Vergata 135, 00133 Roma, Italia. FAX: +39-06-7259-6172. e-mail: defelici@uniroma2.it

0214-6282/2001/$25.00
© UBC Press
Printed in Spain
www.ijdb.ehu.es
efficent purification of early mouse PGCs was also obtained using transgenic mice expressing lacZ under the control of the Akp2tm1Sor mutant allele of the tissue nonspecific alkaline phosphatase (TNAP) and fluorescence activated cell sorter (FACS). It was curious that Anne visited my laboratory in Rome the same year we published our paper on the magnetic cell sorter purification of mouse PGCs. She was happy about our results and eager to know and see all the procedures of our new method. That completed one of the purposes of our original research project on PGCs. However, the other one, the in vitro culture of isolated PGCs, remained unfulfilled.

I remember the frustration in seeing PGCs invariably undergo rapid degeneration whatever culture condition, media and substrates we used. However, when I reported my failure to Anne on her return from her frequent participation in meetings and congresses, she had always words of encouragement and was able to see a positive side to such negative results. The main conclusions of these early studies are well summarised in the sentences we wrote in publishing our first paper on PGC culture (De Felici and McLaren, 1983):

“*In their normal environment, PGCs do not at any time exist as an independent tissue, but are always closely associated with other cells from which they may derive nutrients as well as developmental signals. Such factors may prove critical in controlling the survival of germ cells in vitro and perhaps also their proliferation and development in vivo.*”

Such prediction turned out to be true and some of these factors have now been identified. Although conditions for culturing isolated PGCs have not been achieved yet, the use of feeder layer culture for student in my laboratory, working with Peter Donovan’s group at Frederick, showed, simultaneously with other groups, that SCF is essential for PGC growth in vitro, and that the PGC life-supporting factor discovered in my laboratory in Rome confirmed and extended these results. In particular, we showed that recombinant mouse SCF added to PGCs growing on their own gonadal somatic cells, significantly increased the number of PGCs without directly stimulating their proliferation (Dolci et al., 1991). Immediately afterwards, Matsui et al. (1991) demonstrated that SCF is produced by the somatic cells surrounding migratory and post-migratory PGCs, and that the c-kit receptor is expressed on the surface of PGCs.

In 1991 Susanna Dolci, at that time a Ph.D. student in my laboratory, working with Peter Donovan’s group at Frederick, showed, simultaneously with other groups, that SCF is essential for PGC growth in vitro, and that the PGC life-supporting activity of certain feeder cells is partly due to the production of this growth factor, in particular of the SCF membrane-bound form (Dolci et al., 1991; Matsui et al., 1991; Godin et al., 1991). Experiments carried out in my laboratory in Rome confirmed and extended these results. In particular, we showed that recombinant mouse SCF added to PGCs growing on their own gonadal somatic cells, significantly increased the number of PGCs without directly stimulating their proliferation (Dolci et al., 1993). Moreover, antibodies against c-kit receptor inhibited PGC survival on STO cell feeder layers (De Felici et al., 1993). Such prediction turned out to be true and some of these factors have now been identified. Although conditions for culturing isolated PGCs have not been achieved yet, the use of feeder layer culture for PGCs, has led to the establishment of in vitro culture conditions that allow their survival and proliferation, and the identification of survival and/or proliferation factors needed for PGC development (Table 1). The leukemia inhibitory factor (LIF) was the first growth factor discovered in my laboratory in Rome as able to significantly increase PGC survival in vitro (De Felici and Dolci, 1991). A second growth factor found to exert a very important action on PGCs was the stem cell factor (SCF). In 1990, SCF was identified as a novel growth factor ligand for the tyrosine kinase receptor c-kit encoded by the White (W) locus in hematopoietic cell lines. SCF was found to be encoded by the Steel (Sl) locus and produced from two alternatively spliced mRNAs as either transmembrane or soluble forms (for a review, see Besmer, 1991). Immediately afterwards, Matsui et al. (1990) demonstrated that SCF is produced by the somatic cells surrounding migratory and post-migratory PGCs, and that the c-kit receptor is expressed on the surface of PGCs. In 1991 Susanna Dolci, at that time a Ph.D. student in my laboratory, working with Peter Donovan’s group at Frederick, showed, simultaneously with other groups, that SCF is essential for PGC growth in vitro, and that the PGC life-supporting activity of certain feeder cells is partly due to the production of this growth factor, in particular of the SCF membrane-bound form (Dolci et al., 1991; Matsui et al., 1991; Godin et al., 1991). Experiments carried out in my laboratory in Rome confirmed and extended these results. In particular, we showed that recombinant mouse SCF added to PGCs growing on their own gonadal somatic cells, significantly increased the number of PGCs without directly stimulating their proliferation (Dolci et al., 1993). Moreover, antibodies against c-kit receptor inhibited PGC survival on STO cell feeder layers (De Felici et al., 1993). Such prediction turned out to be true and some of these factors have now been identified. Although conditions for culturing isolated PGCs have not been achieved yet, the use of feeder layer culture for

**TABLE 1**

**MAJOR EVENTS IN THE DEVELOPING OF METHODS FOR PURIFICATION AND CULTURE OF MOUSE PRIMORDIAL GERM CELLS**

(For references see text).

<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>De Felici and McLaren: culture of PGCs purified by EDTA/Percoll gradient</td>
</tr>
<tr>
<td>1986</td>
<td>De Felici et al.: culture of PGCs on Sertoli and follicle cell feeder layers</td>
</tr>
<tr>
<td>1987</td>
<td>Donovan et al.: culture of PGCs on STO cell feeder layers</td>
</tr>
<tr>
<td>1991</td>
<td>Dolci and Dolci: LIF sustains the survival of PGCs in culture</td>
</tr>
<tr>
<td>1991</td>
<td>Matsui et al.: SCF is required for PGC survival in culture</td>
</tr>
<tr>
<td>1992</td>
<td>Matsui et al.; Resnick et al.: production of embryonic germ cells (EG cells) from PGCs in culture</td>
</tr>
<tr>
<td>1993</td>
<td>De Felici et al.: stimulation of PGC proliferation in culture by cAMP-dependent pathways</td>
</tr>
<tr>
<td>1995</td>
<td>Pesce et al.: SCF and LIF promote PGC survival in culture by suppressing apoptosis</td>
</tr>
<tr>
<td>1995</td>
<td>Pesce et al.: SCF and LIF promote PGC survival in culture by suppressing apoptosis</td>
</tr>
<tr>
<td>1996</td>
<td>Capel et al.: establishment of putative immortalized PGC cell lines</td>
</tr>
<tr>
<td>1996</td>
<td>Abe et al.: purification of PGCs from TNAP-lacZ mouse embryos using FACS-gal</td>
</tr>
</tbody>
</table>
Felici, unpublished observations). Most importantly, we found that the addition of SCF to the culture medium markedly reduced the number of apoptotic PGCs during the first hours of culture (Pesce et al., 1993). More recently, we have shown that the effect of SCF on PGC apoptosis is, at least in part, due to a reduction of the expression of the pro-apoptotic gene bax (De Felici et al., 1999). The finding that PGCs begin to undergo apoptosis a few hours after isolation from the gonadal ridges, gave finally an explanation for their rapid degeneration in culture. Clearly, PGCs must be prevented in the embryo from undergoing apoptosis by soluble factors and/or direct contact with the surrounding somatic cells. Interestingly, we have recently reported that the membrane form of SCF also promotes PGC adhesion to somatic cells (Pesce et al., 1997). Most of these results on PGC apoptosis were the object of my presentation at the CIBA Foundation Symposium on Germline Development held in 1993 in London and chaired by Anne McLaren. Some years earlier Anne had invited me to the ESHRE Congress in Paris to present the results about the important role that CAMP plays in stimulating PGC proliferation in vitro (De Felici et al., 1993).

Thus, two aspects of PGC development, their strict dependence on growth factors for survival and a CAMP-dependent pathway for proliferation, were eventually discovered thanks to in vitro culture studies.

Acknowledgments

The research described in this paper was partly supported by MURST National Project “Development and Differentiation of Germ Cells”.

References


DOLCI, S., WILLIAMS, D.E., ERNST, M.K., RESNICK, J.L., BRANNNAN, C.I., LOCK,


