Human Reproduction Update Advance Access published January 30, 2006

 doi:10.1093/humupd/dmk001

Spermatogonial stem cells: questions, models and perspectives

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This review looks into the phylogeny of spermatogonial stem cells and describes their basic biological features. We are focusing on species-specific differences of spermatogonial stem cell physiology. We propose revised models for the clonal expansion of spermatogonia and for the potential existence of true stem cells and progenitors in primates but not in rodents. We create a new model for the species-specific arrangements of spermatogenic stages which may depend on the variable clonal expansion patterns. We also provide a brief overview of germ cell transplantation as a powerful tool for basic research and its potential use in a clinical setting.

Key words: germ cell transplantation/phylogeny/species-specific differences/spermatogonial stem cells

The evolutionary context for the appearance of spermatogonial stem cells

The first large multicellular organisms lived in an aquatic environment of huge dimensions, in which they could move *ad libitum*. Therefore, during sexual reproduction, the chance for a large, immotile female gamete and a small male gamete to encounter each other in the context of external fertilization was low. The most straightforward solution to this problem was the production of huge numbers of motile male sperm. Although each individual male gamete had only a minute chance of fertilizing an oocyte, the vast numbers of male gametes drastically increased the chance for each individual female gamete to be fertilized (Parker *et al*., 1972). This need for high numbers of male gametes was the obvious trigger for the establishment of a stem cell system in the male germ line. This incorporation of a stem cell system has apparently been so highly successful, that it has been maintained throughout evolution and is still present in all recent vertebrates, resulting in the cell type which today is referred to as the spermatogonial stem cell.

Defining primate spermatogonial stem cells

Adult stem cells in mammals are defined by their function (Robey, 2000). All share at least the two following characteristics: (i) they are capable of 'indefinite' self-renewal, meaning that they show usually low mitotic activity throughout the lifetime of the organism without entering differentiation and (ii) they produce differentiating daughter cells (Leblond, 1964). In most stem cell systems, the stem cells do not derive finally differentiated cells directly but do so through progenitor cells. These progenitors are intermediate cell populations inserted between stem and differentiated cells. Such progenitors are well known from the hematopoietic system. They cannot generate new stem cells. Progenitors, however, share some characteristics with stem cells: they are capable of maintaining their own population by self-renewing divisions, and they produce the differentiated cells. But in contrast to the stem cells which show low mitotic activity to generate small numbers of progenitors, the progenitors show a very high mitotic activity and produce the finally differentiated cells needed for tissue homeostasis (Holtzer, 1978). Thus, the stem cells play the role of a regenerative reserve, which under normal healthy conditions is almost mitotically quiescent and shows higher proliferation indices only after major pathological events that have depleted the differentiated cell populations. The progenitor cells play the role of a functional reserve, producing exactly the number of differentiating cells needed for routine tissue homeostasis.

In some systems, e.g. the hematopoietic system, an additional task of the progenitor cells is to pre-determine the later fate of the differentiated cells by entering one of several possible lineages (Bianco *et al*., 1999, 2001; Domen and Weissmann, 1999). In the male germline, though, the main function of the progenitor cells is the high output of differentiating daughter cells, which is obligatory for the continuous daily production of millions of motile sperm.

The spermatogonial stem cell system of the rhesus monkey is well known (Clermont and Leblond, 1959; Clermont, 1972; de Rooij *et al*., 1986, 2002; van Alphen and de Rooij, 1986; van Alphen *et al*., 1988a,b; Marshall *et al*., 1995; Ramaswamy *et al*.,

2000; Bansode *et al*., 2003; Ehmcke *et al*., 2005a,b) (Figure 1). In these macaques as well as in men (Clermont, 1966a,b), two morphologically distinguishable types of spermatogonia exist, the A_{dark} and the A_{pale} spermatogonia. Although both are commonly referred to as spermatogonial stem cells, their biological functions are very different and the A_{dark} shows characteristics indicating that it acts as testicular stem cell. The $A_{pale,}$ however, shows typical characteristics of a progenitor. In healthy adult macaques, the A_{dark} show very low-labelling indices (below 1%). In contrast, the A_{pale} proliferate at defined periods during each cycle of the seminiferous epithelium and produce both A_{pale} and B spermatogonia (Ehmcke *et al*., 2005a,b). In contrast, after cytotoxic insult, the A_{dark} show high labelling indices, apparently when most A_{pale} and B spermatogonia have been destroyed and need to be replenished (van Alphen *et al*., 1988b). A_{dark} also show high-proliferative activity during prepubertal testicular development when the pool of both types of A spermatogonia is expanding (Simorangkir *et al*., 2005). Therefore, the A_{dark} spermatogonium has been recognized as the 'true' testicular stem cell, the regenerative reserve. Its low mitotic activity under normal conditions is very likely favourable for the preservation of genome integrity in the germ line. In contrast, the A_{pale} must be considered the male germline progenitor, the functional reserve. Similar to other precursors the A_{pale} is the cell type through which cyclic proliferation maintains its population and leads to the production of a high number of finally differentiating daughter cells which are needed for the daily production of millions of motile sperm in adult males. The combination of a true stem cell with low mitotic activity and a progenitor producing high numbers of differentiating daughter cells seems to be the ideal system in the male germ line of primates, where both the maintenance of the integrity of the genome and the output of millions of motile sperm are of key importance to insure the potential transmission of the genome to the next generation during a reproductive life that may extend over several decades.

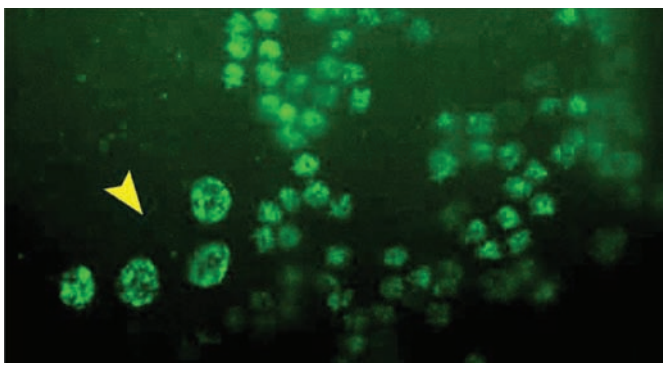

Figure 1. A clone of BrdU-positive A spermatogonia (arrowhead) adjacent to a group of BrdU-positive preleptotene spermatocytes (in S-phase of meiosis) in a whole mount of a seminiferous tubule of an adult healthy rhesus monkey at stage VII of the seminiferous epithelium. The spermatogonia proliferating at stage VII have been identified as A_{pale} in tissue sections (Ehmcke *et al*., 2005). In whole mounts, the nuclei of these BrdU-positive spermatogonia are much larger than the nuclei of the neighbouring preleptotene spermatocytes further indicating that these cells are A_{pale} and not A_{dark} spermatogonia whose nuclei would be of similar size compared with preleptotene spermatocytes.

Differences in the spermatogonial stem cell system in mammals

The types, numbers and the degree of efficiency of spermatogonial stem cell systems vary widely in different species of mammals, and it seems intriguing that self-renewing progenitor populations have, so far, not been detected in all mammals.

Mice and rats

In the mouse, seven types of A spermatogonia (A_{single}, A_{pair}, $A_{aligned}$, A_1, A_2, A_3 and A_4) have been described (de Rooij, 1998; Dettin *et al*., 2003), and their nomenclature has been defined (de Rooij and Russell, 2000). Of those, the A_{single} are considered to be the spermatogonial stem cells. The A_{pair} and $A_{aligned}$ spermatogonia are clonally further expanded colonies which are not synchronized with the seminiferous epithelial cycle. The A_1–A_4 spermatogonia are considered further expansions of these spermatogonial clones which are now synchronized with the seminiferous epithelial cycle. Finally, B and Intermediate spermatogonia are morphologically distinct large interconnected cohorts of spermatogonia which are present at defined spermatogenic stages. Despite minor disagreements about the modalities of spermatogonial expansion in rodents, it is widely accepted that the A_{single} spermatogonia are the only self-renewing spermatogonia in mouse and rat testes (Clermont and Bustos-Obregon, 1968; Huckins and Oakberg, 1978; Hess, 1990). All other spermatogonial subtypes derive via clonal expansion through incomplete mitosis (persistence of cytoplasmic bridges) which in consequence leads to expanding chains of spermatogonia. These spermatogonial subtypes do not undergo self renewal. In this expansion model, the A_{single} spermatogonia are the only renewing germ cells and have to fulfil the tasks of both the regenerative and the functional reserve. Thus, in the rodent, no separate populations of stem cells and progenitors exist. All differentiating germ cells are derived clonally and directly from a single testicular stem cell. However, the large number of subsequent mitotic steps supply rodents with a highly efficient germ cell generating system. In the absence of a progenitor all germ cells derive from an initial stem cell division, however, the task of generating uncountable numbers of sperm can still be achieved with a rather low turnover rate of spermatogonial stem cells in the rodent testis.

Figure 2 illustrates the expansion model and shows the theoretical number of mature germ cells which can be achieved from a single stem cell/progenitor division. The exact mechanism how differentiating progeny is derived from stem cells is unknown, and the existence of unequal divisions is unresolved in the rodent testis. However, with respect to population size each dividing A_s spermatogonium will generate one germ cell committed to become sperm. This, however, does not necessarily mean that these cells divide unequally. It could also be that a complete postmitotic separation of A_s spermatogonia generates two new stem cells, whereas an incomplete separation leads to commitment into the differentiation pathway. The first recognizable differentiating germ cells are the A_{pair} spermatogonia which most obviously derive from an incomplete A_s division and thereafter pass eight mitotic steps before entering meiosis at the spermatocyte stage. In theory, 1024 spermatocytes and thus 4096 haploid spermatids can be generated from each A_{single} spermatogonium entering differentiation in mice and rats.

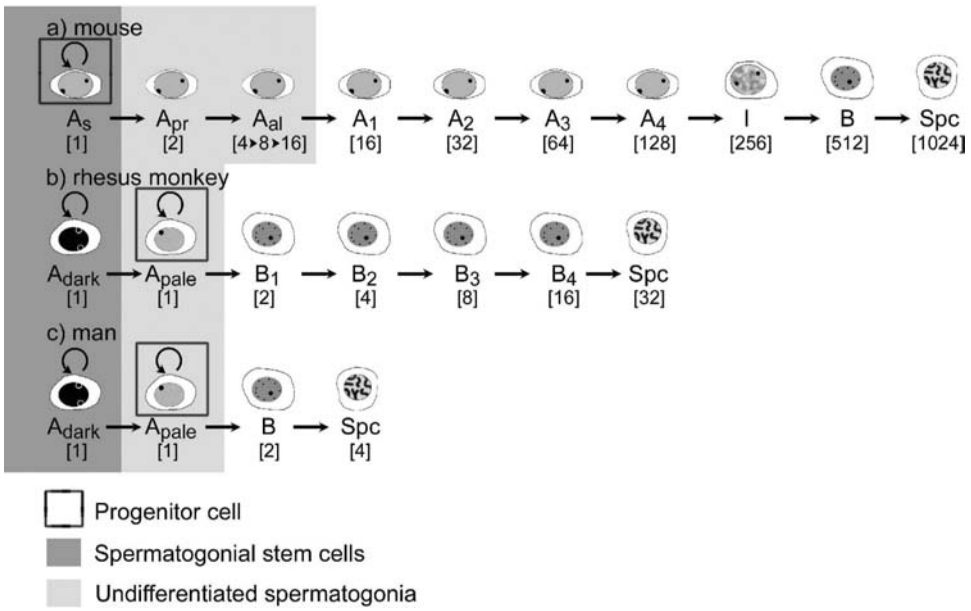

Figure 2. Schematic overview of the premeiotic steps of spermatogenesis in different species of mammals. The number given in brackets underneath the cells indicates the total number of daughter cells derived from any one progenitor cell that enters differentiation.

Rhesus monkey

In the rhesus monkey, the situation is different. The two types of A spermatogonia are the A_{dark} and the A_{pale} spermatogonia (Clermont and Leblond, 1959; de Rooij *et al*., 1986; Ehmcke *et al*., 2005a,b; Simorangkir *et al*., 2005) which are accompanied by four types of differentiating spermatogonia, the B_1, B_2, B_3 and B_4 spermatogonia. As described above, the A_{pale} spermatogonia in this species are self-renewing and thus function as progenitors, whereas the A_{dark} spermatogonia are testicular stem cells and function as regenerative reserve. In this species, it requires five mitotic steps to produce spermatocytes from an initial division of the progenitor. Therefore, a minimum of 32 spermatocytes and thus 128 haploid spermatids can be produced clonally from any progenitor cell in this species (Figure 2). The efficiency of spermatogenesis of other non-human primate species is similar (Wistuba *et al*., 2003).

Human

In the human, although the distinction of two different types of spermatogonia is similar to the monkey (Clermont, 1966a,b), efficiency of clonal expansion is even lower (Bustos-Obregon *et al*., 1975; Johnson, 1994; Johnson *et al*., 1999, 2001). Here again, the A_{dark} spermatogonia function as regenerative reserve and the A_{pale} spermatogonia as progenitors. The latter are followed by only one generation of B spermatogonia before the derivation of spermatocytes. Therefore, theoretically only two mitotic steps occur before the production of spermatocytes from the progenitor cells in the human testis (Figure 2). Only four spermatocytes, leading to the production of 16 spermatids, are thus derived from each initial division of a germline progenitor.

Evolutionary playground: stem cells only or stem cells and progenitors

In all species, a small population of testicular stem cells functions as regenerative reserve and has enormous capacity for the recolonization of the seminiferous epithelium. The main task of this mitotically inactive cell population is the protection of genome integrity and recovery of the seminiferous epithelium after a gonadotoxic insult. It appears that in rodents, the turnover of A_{single} spermatogonia is quite low as the number of mitotic steps allows enormous clonal expansion of germ cells. Therefore, rodents have no need for a precursor in the male germline, and A_{single} spermatogonia function as both reserve cells and progenitor cells. This, however, is different in primates. To generate the same number of germ cells, albeit fewer mitotic steps during germ cell differentiation would need an enormous increase in the mitotic activity of stem cells. In consequence, the higher mitotic turnover subsequently increases the risk for germline mutations and the vulnerability to cytotoxic events. To minimize this risk, a distinct population of progenitor cells is present in the testis of human and non-human primates which take care of the generation of germ cell precursors. The role of stem cells in the primate testis is reduced to the replenishment of precursors in case of cytotoxic or natural depletion.

It is interesting to note that these differences in testicular physiology correlate directly with marked differences in life expectancy and offspring number. It may well be, that, because of the short lifespan of small rodents and the high number of offspring produced during life, the integrity of the germline genome and thus the capability of any single individual male to produce (mostly) healthy offspring has not exerted sufficient selective pressure during phylogeny to implement a true progenitor in the male germline. Starting spermatogenesis always from a stem cell with many subsequent premeiotic divisions leading to large clonal expansion created an obviously appropriate balance for new genetic recombination in rodent species driven by high sexual recombination and a frequent spontaneous appearance of mutations through the male germ line. In contrast, primates have a long lifespan and a relatively low number of offspring per individual. The protection of reproductive capability over a long lifespan leading to intense environmental exposures and the generation of healthy offspring

which is primarily related to an uncompromised integrity of the germline genome are different from rodent species. These different requirements must have been key factors during phylogeny to implement differences in the physiology of testicular stem cells and introducing self-renewing progenitors in addition to stem cells into the male germ line.

Clonal basis of stages of the spermatogenic epithelial cycle

Because of the original description of spermatogenic waves in a number of species, scientists have used the stages of the spermatogenic epithelial cycle to dissect the complex and continuous process of spermatogenesis into smaller, morphologically defined units (Clermont, 1972). The definition of these stages is determined by the observer and relates to morphologically recognizable changes during germ cell development, primarily those of acrosomal changes in round spermatids, and has *per se* no functional relevance for the occurrence of physiological changes. The kinetics of germ cell development are very tightly regulated. Specific functional events are therefore correlated to defined associations of germ cells respective stages of the spermatogenic epithelial cycle. (Hess, 1990). For example, it was possible to define the species-specific starting points of germ cell differentiation and premeiotic divisions to specific spermatogenic stages (mouse: appearance of A_1 spermatogonia: stage V and first division of A_1 spermatogonia: stage IX).

Whereas in rodents and macaques, spermatogenic stages show a longitudinal arrangement, defined by the fact that most tubular cross-sections show only one stage of spermatogenesis, in man and new world monkeys various stages of spermatogenesis are observed in each tubular cross-section. Although researchers have studied comparative aspects of spermatogenic stage arrangements (Wistuba *et al*., 2003; Luetjens *et al*., 2005), the exact cellular mechanisms leading to these differences have not been addressed as yet. Figure 3 depicts a potential model to explain differences leading to longitudinal versus mixed arrangements of spermatogenic stages. If—like in humans—only one division of spermatogonia is encountered before these cells enter meiosis, a very small clone is formed from this initial cell containing a maximum of eight sperm (Figure 2). Because tubular cross-sections with active spermatogenesis contain many more than eight spermatids, several clones must develop at the same time in each tubular area represented in a cross-section. In such a scenario, a longitudinal arrangement can only be achieved when the initial divisions of several progenitors are highly synchronized. It appears, however, that in the human, the initial divisions are not synchronized and start subsequently (helical arrangement) or randomly (random arrangement) resulting in different spermatogenic stages in each cross-section. In contrast, rodent germ cells undergo several divisions as differentiating spermatogonia leading to the establishment of large, interconnected and highly synchronized germ cell clones. An additional clonal enlargement occurs due to the fact that the differentiating divisions of A_1 spermatogonia are initiated from 16 cell clones of undifferentiated spermatogonia. A large number of cells belonging to one clone render it most likely that in any given cross-section most of the germ cells belong to a single clone initiated from a single A_s spermatogonium. Therefore, the longitudinal arrangement of spermatogenic stages in a rodent testis is a consequence of the enormous clonal expansion (Figure 2). Interestingly, the high synchrony of spermatogenesis in recolonized areas following germ cell transplantation confirms the notion that the synchrony of germ cell development is initiated from a stem cell. These colonies are reconstituted from a single undifferentiated spermatogonium leading to large areas populated by highly synchronized germ cells representing a single stage of spermatogenesis (Ventela *et al*., 2002).

A different situation to man and mouse exists in the macaque. Three divisions of differentiating germ cells generate rather small clones (maximally 32 spermatids) of highly synchronized germ cells. However, at each unit area, several of the progenitors start relatively synchronously with their initial division. Because these progenitors are usually two or four cell clones, we estimated that

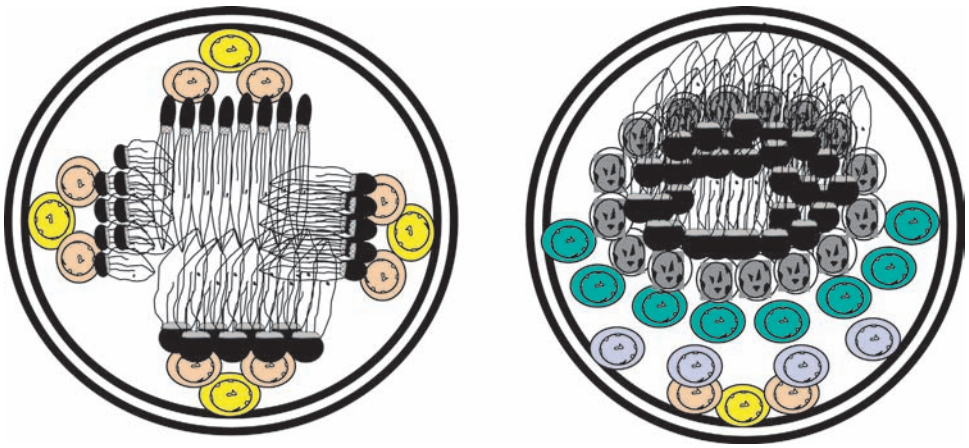

Figure 3. Schematic model for the clonal expansion of germ cells leading to spermatogenic stages in small foci of the seminiferous tubules as seen in the human (left) or to longitudinal arrangements of spermatogenic stages as seen in many rodents and macaques (right) (Luetjens *et al*., 2005). Various generations of differentiating spermatogonia are shown in different colours. Only the elongating spermatids, but no round spermatids or spermatocytes are shown. (Left) If only one division occurs in differentiating spermatogonia (=progenitor), each clone of spermatids arising from this initiating division would maximally generate eight spermatids. In this scenario, a non-coordinated initiation of germ cell development would result in several germ cell clones in any cross-section of a seminiferous tubule to present a different stage of spermatogenesis. (Right) If four divisions of spermatogonia are encountered the progeny from one initial division represents a large clone of 16 cells entering meiosis and finally giving rise to 64 spermatids (the number in the figure is lower to allow easy viewing). In this scenario, large clones of highly synchronized germ cells represent one stage of spermatogenesis in each cross-section of a seminiferous tubule.

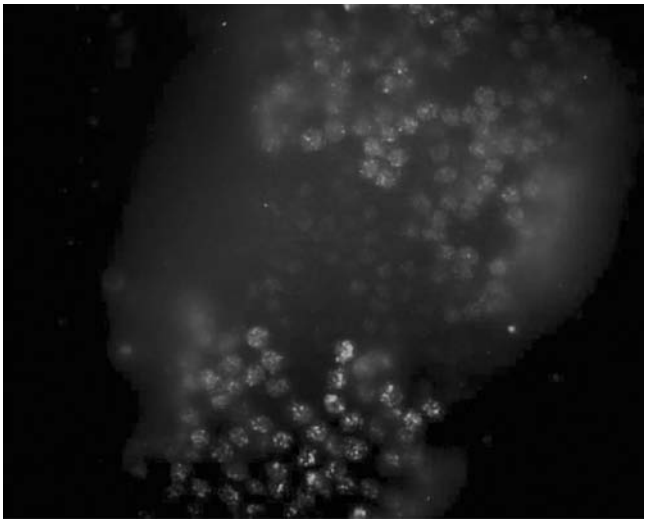

Figure 4. Large clones of BrdU-positive preleptotene spermatocytes are observed in whole mounts of seminiferous tubules of rhesus monkeys 2 h after BrdU administration. In between two intensively BrdU-positive cohorts of germ cells, a cohort of less intensely labelled cells can be seen. We interpret the presence of differently labelled cohorts as the cells seen in this section of a seminiferous tubule belong to three independent clones of differentiating germ cells. Whereas each clone develops in high synchrony as shown by the identical nuclear pattern of BrdU incorporation in all nuclei of a single clone, small time differences in the initiation or termination of germ cell clones leads to different BrdU-staining patterns between each clone as seen in the micrograph.

the average number of cells initiating spermatogenesis synchronously is 16 in the monkey testis (Ehmcke *et al*., 2005a). The fact that several small spermatogonial clones initiate spermatogenesis at the same time leaves the impression of a large clone developing synchronously and with regard to morphological aspects creates a longitudinal arrangement of spermatogenic stages similar to rodents. Figure 4 shows a micrograph of BrdU-labelled preleptotene spermatocytes. Among the large numbers of preleptotene spermatocytes we regularly observe cohorts which are less intensively labelled indicating that they are not in full synchrony with the surrounding cells at the time of BrdU labelling. We interpret this finding as a proof that several clones of germ cells are developing in parallel at any given area but that their initiation is not always fully synchronized.

We conclude that the different mechanisms of germ cell expansion influence the arrangement of spermatogenesis in the seminiferous epithelium. In rodents, the many subsequent divisions following the original stem cell division generate very large clones of cells which take so much space that a longitudinal arrangement of stages is observed. A reduction of differentiating divisions and the introduction of a progenitor cell lead to the generation of smaller germ cell clones. Depending on the size and the number of clones which are synchronously stimulated to initiate spermatogenesis, each spermatogenic stage contains many cells and spermatogenic stages are arranged in a longitudinal fashion or contains only few cells and spermatogenic stages appear as mixed arrangements.

Pluripotency of spermatogonial stem cells

In recent years, exciting new findings have been described showing a high transformation potential of germline cells. Embryonic stem cells and embryonic germ cells are capable to form oogonia and follicle-like structures (Hubner *et al*., 2003). Primordial germ cells isolated from the embryonic epiblast or cells isolated from teratocarcinomas have the potential to colonize the testis and generate male germ cells (Nayernia *et al*., 2004; Chuma *et al*., 2005). Gonocytes derived from postnatal mice transform into embryonic stem cell-like cells when exposed to specified *in vitro* conditions (Kanatsu-Shinohara *et al*., 2005). These and many other findings indicate that the differentiation potential of a germline cell depends on the specific microenvironment and is not cell type specific, although scientists gave the different stages of germ cells different names. The potential for transformation points to the importance of the stem cell niche. Any stem cell is defined by its function. Its specific function, however, is depending on the niche. Falling out of the niche leads to either quiescence or differentiation. This is the reason why numbers of spermatogonial stem cells can only be defined by germ cell transplantation as a functional assay where the cells are introduced into an organ with many unsettled niches, and the ability to settle in these niches is used to determine stem cell numbers. Using markers for the recognition of stem cells is a powerful tool but can be misleading as the size of a true stem cell population in an organ is not defined by the stem cell itself but by the number of niches available for cells which potentially can respond to this microenvironment. It could, therefore, well be that the number of potential stem cells is much higher than the number of niches. In that case, any stem cell isolation attempt shows much higher numbers of stem cells compared to the active stem cell population in the organ. A male germline stem cell must, therefore, simply be defined as any cell which responds to the testicular stem cell niche by obtaining a spermatogonial phenotype and spermatogonial behaviour. This can potentially be many types of cells isolated from the early embryo, tumours or even bone marrow or brain. Typically, germline cells would enter a tumourigenic pathway developing into teratocarcinoma or seminomas under poorly defined micro-environmental conditions or would alternatively enter meiosis under most *in vitro* conditions or when exposed to the ovarian microenvironment. Interestingly, *in vitro* maintenance of male diploid germ cells alone or in co-culture with feeder cells does not lead to the generation of elongating male gametes, although in some studies several markers indicate some degree of meiotic progression (Creemers *et al*., 2002; Feng *et al*., 2002; van Pelt *et al*., 2002; Geijsen *et al*., 2003; Nagano *et al*., 2003). Transplantation of the male germ cells back in the testis allows, however, the generation of male gametes (Nagano *et al*., 2003; Toyooka *et al*., 2003). We conclude from these findings that a variety of different totipotent and germline cells are capable of inducing meiosis under *in vitro* conditions but that male germ cell differentiation is exclusive to the intact testicular microenvironment. Only in the testis, germ cells undergo mitotic arrest in the embryo, develop into a new type of stem cells and undergo the highly complex differentiation into spermatids. In future studies, the transformation potential of germline and pluripotent cells into spermatogonia, primordial germ cell, embryonic stem cells, embryonic germ cells or teratocarcinoma cells will be explored. However, because undifferentiated spermatogonia are diploid germline cells, they should be considered potentially totipotent. The molecular and cellular mechanisms which block spermatogonia under normal circumstances or even after isolation *in vitro* from entering any of the tumourigenic or alternative germline

differentiation pathways are unknown but seem to be related either to the testicular microenvironment or to intracellular mechanisms disabling these germ cells to proliferate without appropriate and specific signal mechanisms.

Potential clinical applications

Transplantation of spermatogonial stem cells was first demonstrated as an assay for stem cell function by Brinster and Avarbock (1994).

In general, the method approaches germ cell development, characteristics and interactions between stem cells and their somatic niches (for review, see Brinster, 2002). Furthermore, the possibility to follow up the donor stem cell in a recipient testis offered insights into the clonal development and the potential of colonization of testicular stem cells (Shinohara *et al*., 2001; McLean *et al*., 2002, 2003; Zhang *et al*., 2003). Besides these basic features, the method was applied as a novel tool to generate transgenic progeny (Ohta *et al*., 2000; Orwig *et al*., 2002; for review, see Wistuba and Schlatt, 2002; Kanatsu-Shinohara *et al*., 2004).

A permanent and complete loss of germ cells is often observed in male patients following oncological therapy. The mechanisms leading to germ cell depletion and the effect of various treatment regimens have been reviewed (Meistrich, 1993; Meirow and Schenker, 1995; Meistrich *et al*., 2003; Howell and Shalet, 2005). Because cytotoxic treatment results in the depletion of the most sensitive premeiotic germ cells, the spermatogenic process has to be reinitiated from the surviving spermatogonial stem cells. Whether spermatogenesis is restored or not depends on the availability and the integrity of these stem cells as well as on the existence of functional stem cell niches. Depending on the dose of cytotoxic treatments supplied during oncological therapy, the stem cell pool is significantly depleted. These patients do not show spontaneous spermatogenic recovery, and if they did not cryopreserve a semen sample before the treatment, the only remaining treatment option for tumour survivors is assisted fertilization with a rather low chance of 22% to become a father (Chan *et al*., 2001). It appears, however, that the somatic environment is not damaged in many of these patients as Leydig cell function is often normal. Also, late recovery of spermatogenesis in some of these patients shows that most likely it is not Sertoli-cell function disallowing spermatogenesis to restart but rather the slow and time-consuming process of spermatogonia to recolonize the seminiferous tubules.

The opportunity to isolate spermatogonial stem cells from oncological patients before oncological therapy and to cryopreserve and reinject them after successful treatment is considered a new option to achieve cure of the patients' infertility and to enable natural conception (Orwig and Schlatt, 2005). This is clearly advantageous to cryopreservation of sperm which is only a reserve and needs to be combined with artificial reproduction techniques. However, considerable risks of transmitting tumour cells back to the patient and many unresolved issues like germ cell retrieval, cell sorting and preservation, efficient and non-invasive techniques for germ cell injections have to be resolved before this strategy can be used in the clinical setting. Many studies have been performed addressing optimal culture conditions for long-term survival of spermatogonial stem cells (Nagano *et al*., 1998, 2002, 2003; Kubota *et al*., 2004). Several studies showed the risk of tumour transmission and reported different outcomes of cell separation to avoid the remission of cancer (Jahnukainen *et al*., 2001; Fujita *et al*., 2005). Magnetic cell sorting could also offer an option for enrichment, but as for the other approaches, this must still be considered an experimental tool (von Schönfeldt *et al*., 1999; Buageaw *et al*., 2005).

In recent years, the potential to generate human sperm by the xenotransplantation of spermatogonia into mice testes was explored. As the sequence and organization of spermatogenesis shows many similarities in all recent mammals, it appeared possible that human spermatogenesis could be initiated in a mouse host, and the initial results after the xenotransplantation of rat spermatogonia into mouse testes were encouraging (Ogawa *et al*., 1999). However, it was later shown that apart from transfers between rodents, cross species transplantation failed achieving complete spermatogenesis (Dobrinski *et al*., 1999, 2000; Reis *et al*., 2000; Nagano *et al*., 2001, 2002). Interestingly, spermatogonial stem cells of all species settled in the mouse testis indicating highly conserved mechanisms of stem cell recognition and spermatogonial niche occupation. However, the studies revealed that evolutionary differences in the regulation of spermatogenesis do not allow the xenodifferentiation of germ cells, most likely because of disturbed communication between non-rodent germ cells and a mouse seminiferous epithelium. This inability of non-rodent spermatogonia to initiate full spermatogenesis in a mouse testis may very well also be related to the crucial differences in the spermatogonial stem cell/progenitor systems we have discussed here. It could be related to the inability of the mouse testis to support the function of the progenitor population required by primate spermatogenesis for normal function.

Enthusiasm for the therapeutic potential of germ cell transplantation was enhanced by our work (Schlatt *et al*., 2000), which demonstrated the feasibility of transplanting germ cell suspensions into the testes of non-human primates and dissected testes from men. A preclinical study using macaques whose testes had been germ cell depleted by local irradiation (Schlatt *et al*., 2002) highlighted many of the challenges that will be encountered when this technique is applied to patients. Thus, some of the crucial steps for successful refertilization are the safe retrieval of sufficient testicular tissue before the cytotoxic insults, avoidance of ischemia, cryopreservation and thawing of cell suspensions or tissue, sorting of tumour cells or enrichment of stem cell spermatogonia, and efficient ultrasound guided non-invasive transfer of germ cell suspensions into the *rete testis*. Responsible long-term development of the transplantation technique in non-human primates that model the reproductive deficits of cancer survivors will provide new insights in an animal system that has relevance for human physiology. The results will be instructive for future clinical trials.

In conclusion, germline transplantation is a powerful tool for the study of spermatogonial stem cells and the role of germline and somatic cells in the testis, but a future use in a perspective clinical setting definitely requires many more studies proving the efficiency and safety of this approach.

Acknowledgements

Our own work was financially supported by NIH grants RO1 050617-01 and R12 AG 024914-01.

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Submitted on October 10, 2005; resubmitted on November 17, 2005; accepted on December 20, 2005