Study on the mechanism for exclusion of degenerating spermatogenic cells: membrane phospholipid-mediated phagocytosis by Sertoli cells

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学位授与の題目	Study on the mechanism for exclusion of degenerating spermatogenic cells:membrane phospholipid-mediated phagocytosis by Sertoli cells（セルトリ細胞による細胞膜リン脂質を介した精子形成細胞の貧食機構）
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学位論文要旨

In the mammalian testis, more than half of the differentiating spermatogenic cells die most probably by apoptosis and are rapidly eliminated during spermatogenesis. Elimination of degenerating spermatogenic cells is believed to be executed through phagocytosis by testicular somatic cells termed Sertoli cells. However, precise mechanism for this phagocytosis reaction has yet to be elucidated. In this study, an *in vitro* phagocytosis assay was established and phagocytosis of apoptotic spermatogenic cells by Sertoli cells was quantitatively analyzed.

1) Establishment of the phagocytosis assay

Testicular cells from 20-day-old rats were primary cultured, and spermatogenic and Sertoli cells were individually isolated. Most of the spermatogenic cells consisted of spermatocytes in terms of their morphology and ploidy. When these cells were cultured without Sertoli cells, they underwent typical apoptotic death. When the apoptotic spermatogenic cells were labeled and added back to the Sertoli cell culture, phagocytosis by Sertoli cells was clearly detectable under a microscope. The cells with less viability were more susceptible to phagocytosis by Sertoli cells, indicating that Sertoli cells selectively recognized and phagocytosed apoptotic spermatogenic cells.

2) Cell-type specificity in spermatogenic cell phagocytosis

In order to examine whether spermatogenic cells at particular states in

their differentiation were more preferably phagocytosed by Sertoli cells, spermatogenic cells were separated in a density gradient of Percoll and subjected to a phagocytosis assay. The cells with a ploidy of $1n$, $2n$, and $4n$ were almost equally phagocytosed. These results indicated that Sertoli cells phagocytosed apoptotic spermatogenic cells regardless of their differentiation states.

3) Identification of the phagocytosis marker

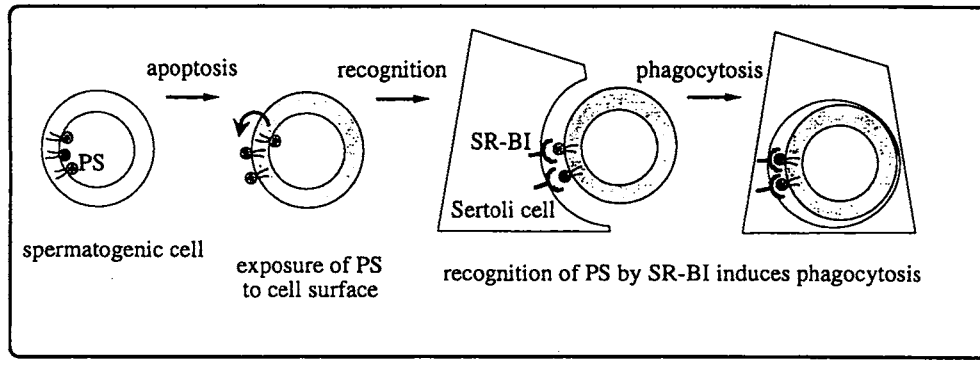
Membrane phospholipids are asymmetrically localized in the two leaflets of the membrane bilayer; for example, phosphatidylserine (PS) and phosphatidylethanolamine (PE) are restricted to the inner leaflet. Such asymmetry disappears in apoptotic cells and phospholipids are likely to be re-distributed evenly in the two leaflets. This may cause cell surface exposure of PS and PE, which would otherwise remain in the cytoplasmic side. In order to examine the possibility that externalized PS and/or PE serve as the phagocytosis marker recognizable by Sertoli cells, their cell surface localization was determined in flow cytometry using specific probes. The results clearly showed that both PS and PE became exposed to the surface of spermatogenic cells when they were cultured without Sertoli cells. All the spermatogenic cell populations separated through a Percoll gradient exhibited exposure of PS and PE. Moreover, phagocytosis reactions of all those cells were severely impaired by the addition of liposomes containing PS, whereas liposomes consisted of PE or other neutral phospholipids showed little effect. The above results indicated that PS translocated from the inner to the outer membrane leaflet in apoptotic spermatogenic cells regardless of their differentiation states and that recognition of PS by Sertoli cells led to phagocytosis of spermatogenic cells.

4) SR-BI as the phagocytosis-inducing PS receptor in Sertoli cells

Class B scavenger receptor type I (SR-BI) has been a strong candidate for the phagocytosis-inducing PS receptor in non-macrophage-type phagocytes. Since SR-BI mRNA was detected in Sertoli cells, its role in recognition and phagocytosis of spermatogenic cells was examined. When a SR-BI cDNA isolated from rat Sertoli cells was introduced into Sertoli-derived culture cell lines, their activity for binding to PS-containing liposomes and phagocytosing spermatogenic cells in a PS-mediated manner significantly increased. These results indicated that SR-BI functioned as the PS receptor and induced phagocytosis of spermatogenic cells by Sertoli cells.

5) Conclusion

I propose a model for selective phagocytosis of apoptotic spermatogenic cells by Sertoli cells as follows. Upon apoptosis induction, PS is externalized on the surface of spermatogenic cells. It is then recognized by SR-BI present on the surface of Sertoli cells. The binding of PS and SR-BI leads to phagocytosis of apoptotic spermatogenic cells by Sertoli cells.



学位論文審査結果の要旨

白土明子から提出された学位論文について，各審査委員による査読の後に平成10年1月28日に口頭発表会が公開で行われた。同日に上記5名の審査委員によって最終の審査委員会が開かれた結果，以下の理由により当該論文は博士（学術）の学位を授与するに値すると判定された。

この論文は，精子形成過程で細胞死を起こした生殖細胞が排除される機構についての研究結果を記述したものである。以前より，精巣内体細胞であるセルトリ細胞が生殖細胞を貪食することが示唆されていた。白土は，ラットおよびマウスの精巣細胞の初代培養系を利用して定量的な貪食実験系を確立し，貪食反応の機構を詳細に解析した。その結果，精子分化のあらゆる段階にある生殖細胞が，細胞表層に出現した膜リン脂質ホスファチジルセリンに依存してセルトリ細胞に貪食されることが示された。さらに，セルトリ細胞のスカベンジャー受容体がホスファチジルセリンを認識して生殖細胞の貪食が誘導される可能性が指摘された。

論文は明瞭に記述されており，各実験におけるデータも信頼性が高いと思われる。ただ，ホスファチジルセリン受容体の証明が残されるなど，真の意味での機構解明がなされたわけではなく，論文の最後に示されているモデルを実証するためにはさらに解析が必要だと思われる。しかしながら，この研究は雄性生殖細胞排除の機構を初めて分子レベルで解析したものであり，得られた成果は哺乳動物精子形成の理解を深めるものと評価される。