行政院國家科學委員會專題研究計畫成果報告

題目(中文)：第十一型介白質與白血病抑制因子於蛻膜化及胎盤形成之角色

題目(英文)：The role of interleukin-11 and leukemia inhibitory factor in decidualization and placenta formation

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中文摘要

屢次著床失敗與習慣性流產的病例一直是臨床上治療生育方面的問題中最難以解決的課題。截至目前爲止，許多這類病人的致病機轉並不清楚，因此即使使用最進步的人工協助生殖技術依舊無法解決許多患者的問題。所幸，最近不斷有許多新的報告顯示這類生育問題可能與胚胎著床與胎盤形成過程中，某些細胞素與生長因子交互作用有密切相關性。這其中尤其以「白血病抑制因子（LIF）」與「第 11 型介白質（IL-11）」特具潛能。

在動物實驗上已經發現，母小白鼠如果缺乏 LIF 基因，則雖然可以製造卵子，形成胚胎，並發育至囊胚，但是這些囊胚完全無法著床。但是如果把合成的 LIF 注入小白鼠腹腔中則能著床。此外如果把這些 LIF-/- 的囊胚植入正常假性懷孕的母鼠中，則也可以著床。因此代表在著床期母體表現出 LIF 基因對於著床有絕對重要性。另外的研究則指出，如果小白鼠的 IL-11 接受體（IL-11R）基因被除掉，則胎盤的形成會受到阻礙，因此胎兒發育受損。因此這些研究顯示 IL-11 與 LIF 之基因表現對於胚胎之順利著床與胎盤形成有其基本的重要性。

因此本研究收集人類的蛻膜與子宮內膜組織，然後使用 real-time PCR 與組織免疫方法確定其是否有 LIF、LIF-R、IL-11 與 IL-11R 的存在，比較其在不同病人（屢次著床失敗與習慣性流產患者）之差異。結果發現 IL-11 與 LIF 相關基因的確在蛻膜與子宮內膜組織有表現，且 IL-11 可以由蛻膜與胚胎組織產生出來。而當基礎的 IL-11R 存在之下，IL-11 產生的不足可能是造成萎縮卵的原因之一。至於 LIF 相關基因

則在胚胎著床之後的胎盤形成過程中，影響較少。

藉此實驗，我們可以進一步瞭解胚胎著床與胎盤形成之調控，以使將來能找出更有效的方法來促進生育之成功。

英文摘要

Previous study demonstrated that leukemia inhibitory factor (LIF) and interleukin-11 (IL-11) are critical factors in the peri-implantation stage. For example, Interleukin-11 receptor α knockout female mice (*IL-11Rα*$^{-/-}$) were phenotypically normal but infertile due to defective decidualization.However, the role of IL-11 signaling in human reproduction remains unclear. This study examined the expression of IL-11, IL-11Rα, and signal transduction factor glycoprotein 130 (gp130) in different phases of endometrium (6 in proliferative phase and 4 in secretory phase), and the decidua and villi of normal pregnancy (NP; n=25) and anembryonic pregnancy (AP; n=25) in the first trimester (gestational week 7-9). RT-PCR showed IL-11, IL-11Rα, and gp130 mRNA expression in all samples, except the absence of IL-11 signal in the unstimulated MRC-5 cell and the proliferative phase endometrium. Real-time quantitative PCR showed that the relative level of IL-11Rα mRNA was not significantly different among proliferative phase endometrium (relative level; mean ± SEM, 1.4 ± 0.5), secretory phase endometrium (1.3 ± 0.1), or decidua from NP or AP (1.7 ± 0.3 and 1.9 ± 0.4, respectively), but was significantly greater in chorionic villi either from NP or AP (7.6 ± 1.3 and 10.6 ± 1.9, respectively; both $P < 0.05$ compared to decidua or endometrium). No difference of IL-11Rα mRNA level was found between NP and AP (1.7 ± 0.3 vs. 1.9 ± 0.4 in deciduas; 7.6 ± 1.3 vs. 10.6 ± 1.9 in villi; both $P > 0.05$). In situ hybridization localized IL-11Rα mRNA expression in proliferative phase endometrium (stroma only), secretory phase endometrium (stroma and gland), decidua (stroma and gland), and villi (trophoblast and stroma). The staining intensities were not distinctly different between different groups of samples, or between different cell types in a sample. No difference in IL-11Rα expression was found between NP and AP when either decidua or chorionic villi was analyzed. IL-11 mRNA level was not detected in the proliferative phase (relative level, 0.0 ± 0.0), barely detectable in the secretory phase (0.03 ± 0.02), and significantly increased in decidua (1.7 ± 0.2 and 0.1 ± 0.1, respectively for NP and AP) and chorionic villi (13.0 ± 2.2 and 0.2 ± 0.1). In addition, IL-11 mRNA level was higher in NP than in AP both in decidua (1.7 ± 0.2 vs. 0.1 ± 0.1 vs. 0.1; $P = 0.03$) and in villi (13.0 ± 2.2 vs. 0.2 ± 0.1; $P < 0.001$). Immunohistochemistry study showed that IL-11 was nearly absent in endometrium in both phases, but clearly detectable in decidua and villi. Consistent with the results of quantitative PCR, the staining intensity was stronger in villi and decidua from NP than those from AP. The spatial and temporal changes in IL-11 and its receptor observed in this study suggest that IL-11 may be produced both by the embryo

(predominantly) and the decidual cells and exerts its action on chorionic villi and decidua in an autocrine or paracrine manner. In the presence of a baseline level of IL-11Rα, IL-11 may subsequently regulate placentation and decidualization for the maintenance of a normal pregnancy. The finding of decreased IL-11 expression in the absence of any change in IL-11Rα in AP suggests that defective expression of IL-11 but not IL-11Rα may account for certain cases of anembryonic pregnancy. These observation have recently been published in the June issue of Journal of Clinical Endocrinology and Metabolism in this year.

On the other hand, we also did similar examination of LIF and LIF-R on these tissues. Preliminary data showed that in contrast to the observation in IL-11-related gene expression, there is no difference of LIF and LIF-R gene expression between normal and anembryonic tissues. Both conventional semi-quantitative PCR and real-time quantitative PCR have been done and both obtained similar findings. We are preparing the manuscript and soon a paper will be submitted.

計劃緣由與目的

The mechanisms underlying failures of implantation or placental development remain mostly unknown. Recently, a number of growth factors and cytokines including colony stimulating factor (CSF), leukemia inhibitory factor (LIF) and interleukin–11 (IL-11) have been suggested to play a role in these processes. A temporal analysis revealed that IL-11 expression was maximal in the normal pregnant uterus at the time of decidualization in mice. Subsequently, mating of female mice with a null mutation of the IL-11 receptor α ($IL\text{-}11R\alpha^{-/-}$) with fertile males of any genotype for periods up to 12 months never resulted in clinical pregnancy. Histological study showed that these female mice were infertile due to defective decidualization. This observation identified a previously unrecognized critical role of IL-11 in female reproduction of mice during the period of decidualization. However the role of IL-11 signaling in humans has not been established. Further studies are therefore needed to evaluate the expression of IL-11 and IL-11Rα in pathological conditions associated with human implantation failure or recurrent abortion.

IL-11 was first isolated as a soluble factor produced by the PU-34 primate bone marrow stromal cell line and subsequently human IL-11 was cloned. The actions of IL-11 are mediated through a receptor complex composed of IL-11Rα and signal transduction factor, glycoprotein 130 (gp130). Recently, IL-11 has been shown to have pleiotropic effects in many tissues, including the hematopoietic system, skin, bone, central nervous system, thymus, and lung. Studies have also suggested that IL-11 plays various roles in the urogenital system . For example, immunoreactive IL-11 was detected in human follicular fluid, though its action in the ovary has not been established.

The present study was first designed to evaluate the role of the IL-11 system in early human pregnancy, especially in

decidualization and placental formation. We hypothesized that gestational tissues from patients with spontaneous abortion might be characterized by differences in traits of IL-11 expression compared to those in normal pregnancy. To test this hypothesis, we analyzed and compared IL-11, IL-11Rα, and gp130 expression in normal pregnancy (NP) and anembryonic pregnancy (blighted ovum; AP).

In addition, Gp130 cytokines, including leukemia inhibitory factor (LIF), interleukin-6 (IL-6), interleukin-11 (IL-11), OSM, CNTF, and CT-1, have been observed to be closely related to human implantation. Expression of leukemia inhibitory factor (LIF) in the endometrium has been found to be absolutely essential for mouse embryo implantation. Similarly in human, the peak expression of LIF and its receptor (LIF-R) in the endometrium at the mid-secretory phase of the menstrual cycle also suggests a potential autocrine and paracrine role for LIF in regulating human embryo implantation. Recently, we further identified the expression of LIF and LIF-R in mouse and human embryos throughout the entire pre-implantation stages. In addition, mice in which the gene encoding LIF-R has been deleted also show abnormal growth and development of the placenta. These above works denote the important role of LIF at the peri-implantation stages. Actually, dys-regulation of LIF expression has been reported in patients with repeated implantation failure.

However, the exact molecular mechanism of LIF action on the embryo, placenta or the decidua remains ill defined. It is therefore interesting and potentially clinically useful to examine the detailed mechanism of LIF action in these sites and stages. This clinical applicability is based on the fact that failure in blastocyst implantation in LIF-/- mice can be completely reversed by infusion of LIF. In addition, LIF-related cytokine, e.g. Interleukin-11 (IL-11) can be used clinically to treat certain diseases. Therefore, we design the present study to find out: [1] the induction and regulation of LIF and LIF-R expression in the peri-implantation and early gestational stages. [2] the mechanism of action of LIF in these tissues (decidua and fetus). Therefore in this study we also examined the expression of LIF and its related genes in the materno-fetal interface.

結果

The expression of IL-11, IL-11R and gp130 genes

PCR revealed that all the samples expressed IL-11 mRNA. These included K562 cells, MRC-5 cells stimulated by IL-1α and PMA (served as positive control), HeLa cells, human PBMC, endometrium in the secretory phase, and decidua and chorionic villi samples from all patients . In addition, IL-11Rα and gp 130 mRNA were detectable in all samples.

Quantitation of IL-11 and IL-11Rα mRNA by quantitative PCR

Real-time quantitative PCR identified

that IL-11Rα mRNA levels did not change in the endometrium during each phase of the menstrual cycle and in the decidua after pregnancy was established. However, chorionic villi contained a distinctly higher level of IL-11Rα mRNA than decidua or other phases of endometrium. There was however no difference in IL-11Rα mRNA level between NP and AP, in either decidua or chorionic villi.

Real-time PCR for IL-11 indicated that IL-11 mRNA level was low in endometrium during the proliferative phase and increased during the secretory phase and after normal pregnancy. In AP, the IL-11 mRNA level also increased compared to that in endometrium of both phases although it was not as high as in NP. The IL-11 mRNA levels were significantly higher in NP than in AP, both in decidua and chorionic villi.

Immunohistochemical study for IL-11

Immunohistochemical found that the immunoreactive IL-11 was barely detectable in the glandular epithelium of secretory phase endometrium and was undetectable in proliferative phase endometrium. After pregnancy however, IL-11 immunoreactivity became detectable both in the stromal and glandular cells of decidua and syncytiotrophoblast and cytotrophoblast cells of the chorionic villi. Stronger immunoreactivity intensity was found in the glandular epithelium than in the stromal cells of decidua. In contrast, the intensity was stronger in trophoblast than in stroma of chorionic villi. Comparison of tissue samples from different groups of patients showed that chorionic villi from NP produced a higher level of IL-11 than that from AP. Similarly, decidua from NP expressed stronger IL-11 immunoreactivity than those from AP.

In situ hybridization for IL-11Rα mRNA

In situ hybridization technique was used to examine the expression of IL-11Rα gene in the endometrium and gestational tissues. Proliferative phase endometrium expressed IL-11Rα mRNA in its stromal cells but not glandular cells. In the secretory phase however, IL-11Rα expression was evident in both stromal and glandular cells. During pregnancy, both stromal and glandular cells of the decidua and trophoblasts of the chorionic villi continuously expressed this transcript. However, there was no distinct difference of staining intensity was found among samples from secretory phase endometrium, decidua, or chorionic villi. There was neither significant difference of IL-11Rα mRNA levels between NP and AP in either decidua or villi.

討論

Implantation and placentation require the synchronized growth of an embryo and a continuous and adequate decidual development in the endometrium. It is conceivable that these processes are susceptible to endogenous or exogenous insults. Among them, defective IL-11Rα action has been shown to be the mechanism for abnormal decidualization in mice. That study reasonably suggests a potential role of

the IL-11 system genes in abnormal decidualization in humans. The present study demonstrated the expression of IL-11Rα mRNA in the endometrium throughout the menstrual cycle and in the decidua of early pregnancy. Although the level of IL-11Rα mRNA in chorionic villi was higher than in decidua, no significant difference in IL-11Rα mRNA levels was found between decidua and chorionic villi from NP and AP. In contrast, the IL-11 mRNA level in endometrium progressively increased from the secretory phase and reached peak levels in the decidua and chorionic villi after pregnancy. IL-11 mRNA levels in both decidua and villi of AP were significantly lower than those of NP. Gp130 mRNA was also detectable in all of the samples examined. These data support the hypothesis that IL-11 signaling in human endometrium and gestational tissue.

The results of our study showed that both IL-11 and IL-11Rα mRNA were present in the endometrium throughout the menstrual cycle and during early pregnancy. It also demonstrated that IL-11Rα levels remained stable during these stages with minimal variation between levels in the endometrium and the decidua. However the real-time quantitative PCR results of our study also showed that IL-11Rα and IL-11 expression were augmented in the chorionic villi compared to the decidua. This suggests that IL-11 may be produced both in the embryo and in decidual cells, though the former may be the predominant source in humans due to its greater level. Subsequently, through binding to IL-11Rα and gp130, IL-11 may exert its action on the embryo and the decidua to regulate chorionic villus development and decidualization, respectively. This potential dual action of IL-11 however was not clearly demonstrated in a previous study by Robb et al in mice which did not unanimously support the role of IL-11 action in trophoblast development. Therefore further study is needed to explain whether the role of IL-11 differs between species.

There are inconsistent results obtained by different experimental methods that need to be noted. As detailed previously in the Results section, in situ hybridization did not show a distinct difference of staining intensity between samples from secretory phase endometrium, decidua or chorionic villi. This data is not consistent with the result of real-time quantitative PCR, which showed that chorionic villi expressed a significantly higher level of IL-11Rα mRNA than decidua. The reason for the divergence of these results is not clear, but may involve varied sensitivity of each of the experimental methods.

The presence of IL-11Rα mRNA with minimal variation in amount despite changing IL-11 levels suggests divergent actions between IL-11 and IL-11Rα. One possible explanation for this finding is that the level of IL-11 rather than IL-11Rα may regulate normal placental formation and decidualization. Although previous data indicate that IL-11Rα knockout leads to infertility in mice, complete IL-11Rα depletion in humans has not been reported.

Therefore, a baseline IL-11Rα level most likely is more than enough to fulfill its role in IL-11 function. This argument is further supported by the lack of difference of IL-11Rα expression between NP and AP. However, the potential for defects in IL-11Rα action due to either mutation or deletion in the IL-11Rα gene cannot be excluded at present. It would therefore be interesting to investigate IL-11 and IL-11Rα at the genomic level in patients with recurrent spontaneous abortion to identify their possible defects in action.

A significant decrease of IL-11 mRNA was found in AP compared to NP. In the presence of IL-11Rα, the level of which might only be rarely under the threshold level in humans, the resulting decreased level of IL-11 might lead to defective placental formation and subsequently result in a blighted ovum (anembryonic pregnancy). However, we cannot completely rule out the possibility that decreased IL-11 level could be secondary to fetal demise, rather than itself being the primary phenomenon. Consequently, more work is needed to clarify this cause-and-effect relationship.

IL-11, IL-6, ciliary neurotrophic factor (CNTF), LIF, and oncostatin M (OSM) belong to a specific group of cytokines that share the common signal-transduction subunit gp130. Overlapping of the biological function of these cytokines may at least partly be explained by the sharing of this common signal-transduction component in formation of their high-affinity receptor complexes. As expected, this study identified the expression of gp130 mRNA in phases of endometrium and in gestational tissues. These data provide an indirect evidence of the role of gp130 in the complete action of IL-11Rα. However further detailed spatial and temporal analyses of this gene will be needed to clarify its role in human disease states.

In conclusion, the present study suggests that IL-11 may play a crucial role in the maintenance of early pregnancy. It is likely that IL-11 has dual actions, both on placentation and decidualization, though in humans the major focus might be on the former. Decreased expression of IL-11 during or after implantation probably can account for some cases with implantation failure or recurrent abortion in early pregnancy. This conclusion however needs further confirmation since the cause vs. effect relation cannot be determined at present. In addition to the evident academic interest, further studies of IL-11 action will have important clinical implications. Medical use of IL-11 has been shown to effectively enhance a number of biological functions including bone marrow recovery and platelet formation. Extrapolation from these experiences provides strong support for its potential use in treating diseases of the genital organs, where IL-11 action has previously been identified. In addition, the medical use of IL-11 is relatively nontoxic compared to other cytokines. Therefore, after further confirmation, it is likely that IL-11 may eventually be shown to have clinical applications in the treatment of repeated implantation failure and/or recurrent spontaneous abortion.

計劃成果自評

Essentially, this study has mostly been conducted according to the original protocol. The part on the IL-11 had been completed and published in this year (J Clin Endocrinol Metab 87;2320-2328, 2002). The part on the LIF especially on the real-time quantitative PCR have also been completed recently and a manuscript about this part is being prepared. Therefore, this two-year study can be rated as favorable as regards to the expected results. We hope that further study in these aspects can be continued and likely more fruitful results can ensue.

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