行政院國家科學委員會專題研究計畫成果報告

蛻膜內巨噬細胞在懷孕初期所扮演的角色(1/3)
The role of decidual macrophages in early pregnancy

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一、中文摘要

哺乳動物的胎兒向來被視為一種成功的半異體移植物，而胎兒能否在母體子宮內正常生長發育，則取決於母體免疫系統之趨向，即是當子宮內微環境以第二型輔助型 T 細胞反應為主時，胚胎才能正常發生；反之，若是以第一型細胞免疫反應為主時，則會干擾胚胎發育。由先前已發表文獻中得知，出現在人類蛻膜中的白血球以巨噬細胞、子宮自然殺手細胞及 T 細胞為主；此外，無論是在人類或是小鼠中，巨噬細胞皆佔了 20~30% 的高比例，大多數學者推測巨噬細胞在蛻膜中扮演了極重要且獨佔的調控角色，例如可能藉由分泌細胞激素或細胞驅動激素等可溶性因子來執行其調控機制，但是仍僅止於推測階段，這群細胞確實的重要性則有待進一步的實驗來證實。本研究中觀得蛻膜巨噬細胞表面表現高量的 CD30L 與 HLA-DR 分子，表示蛻膜巨噬細胞呈現被活化狀態，但是卻僅只表現極微量的 CD80 與 CD86 分子。根據這些發現，我們推測蛻膜巨噬細胞可能是借由 CD30L 與蛻膜基質表現的 CD30 交互作用，進而調控細胞表面 CD80 與 CD86 等活化輔助因子的表現，來抑制母體 T 細胞的正常免疫反應，使得胚胎得以不被母體排斥而正長發育。

關鍵詞：蛻膜中的巨噬細胞、免疫調控、細胞間交互作、CD30、CD30L

Abstract

The mammalian fetus has been considered as a successful semiallograft after implantation and maternal immunomodulation can influence fetal survival. Previous studies showed that macrophages are one of the most abundant leukocytes (around 30% of the decidual leukocytes) in decidua and persist in large numbers throughout gestation. They are found primarily cluster around the implantation sites. Thus, they may play some regulatory roles in early pregnancy. It has been hypothesized that fetal and maternal macrophages are pivotal regulatory cells in the membrane environment. Many functions have ascribed to these cells; for example, decidual macrophages may utilize their mediator products to provide the TH2-like immune state in feto-matenal interface in early pregnancy. However, the accurate role of decidual macrophages remains unclear and needs to be further defined. In the current study, we found there are high levels of CD30L and HLA-DR expression on the surface of decidual macrophages, but low expression of CD80 and CD86 co-stimulatory molecules. Based on the observation, we propose that decidual macrophages are modulated in CD80 and CD86 upon CD30- CD30L interaction.

Keywords: decidual macrophages, immuno-regulation, cellular interaction, CD30, CD30L,

二、緣由與目的

The mammalian fetus has been considered

as a successful semiallograft after implantation and maternal immunomodulation can influence fetal survival. Previous studies showed that not only in human, but also in murine model, macrophages are one of the most abundant cell populations in decidua and persist in large numbers throughout gestation. They are found primarily cluster around the implantation sites. Thus, they may play some regulatory roles in early pregnancy. Although macrophages are one of the major cellular populations in the early pregnant uteri (around 30% of the decidual leukocytes), there are relative few studies discussing about them because of the extreme difficulties in their isolation and purification from the uteri.

It has been hypothesized that fetal and maternal macrophages are pivotal regulatory cells in the membrane environment. Many functions have ascribed to these cells, ranging from the classical immunological roles of antigen presentation, bacteriocidal activities, immunosuperssion and the production of cytokines to phagocytosis. As we know TH2-biased maternal microenvironment is required for successful pregnancy, decidual macrophages may utilize their soluble medi- ators to achieve the local TH2-skewed immune state in feto-maternal interface in early pregnancy. However, the accurate role of decidual macrophages remains unclear and needs to be further defined.

CD30 expression is highly associated with tumor cells, but is has been also found in placenta with normal pregnancy. It implicated that there should be corresponding CD30 ligand (CD30L) adjacent to CD30. Thus it's sensible to suggest that some signal is delivered upon CD30 ligation. There is evidence revealed that reverse signaling appears in CD30-CD30L interaction. Whether there is any CD30L expression and the consequence of the interaction happened in decidua require further investigation. In addition, decidual macrophages derived soluble factors may act on other cells in uteri. The interaction between decidual macro-

phages and neighboring cells is thus also important.

The purposes of this study are to propose the satisfactory hypothesis for the regulatory roles of decidual macrophages in early pregnancy and make efforts to find out a reasonable immunotherapy for the patients with infertility and recurrent spontaneous abortion.

三、結果與討論

After enzyme digestion and 50-μm steel mesh filtration, single cells derived from early pregnancy were determined for surface marker expression by flow cytometer. Live cells were gated and analyzed the level of CD11b and HLA-DR at the same time. As shown in Fig. 1, all CD11b$^+$ cells were HLA-DR$^+$.

It has been implicated that decidual macrophages were activated in situ. Since CD80 and CD86 are also upregulated while macrophages become activated, we further trace the expression of these two co-stimulatory molecules. CD11b$^+$ cells were gated (Fig. 2A, 3A) and analyzed the CD86 or CD86 expression. CD11b$^+$HLA-DR$^+$ were CD80$^-$ CD86^{low}. (Fig. 2B, 3B) This is in coincidence with the previous study that indicated placental macrophages have a decreased ability to present antigen in mouse model. (PNAS. 90:462, 1993)
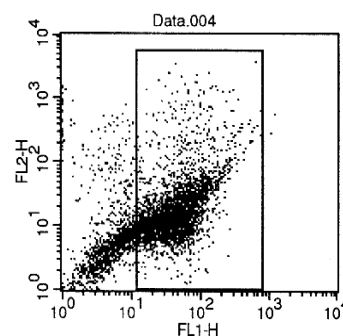

Fig.1 All CD11b$^+$ cell were HLA-DR positive. One million single cells were used for flow cytometry. We gated live

interaction. Thus, we determined the appearance of CD30L on HLA-DR$^+$ cells and found HLA-DR$^+$ cells were CD30L$^+$ and the CD30L was medium expression. (Fig. 4A) Approximate 90% decidual macrophages express CD30L. (Fig.4B)
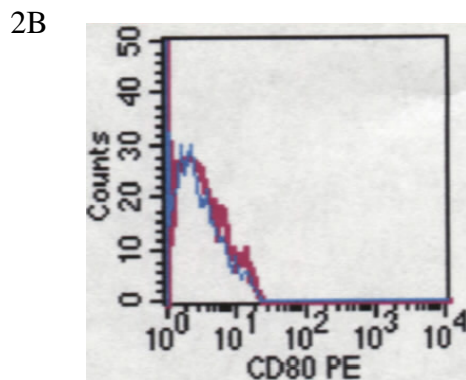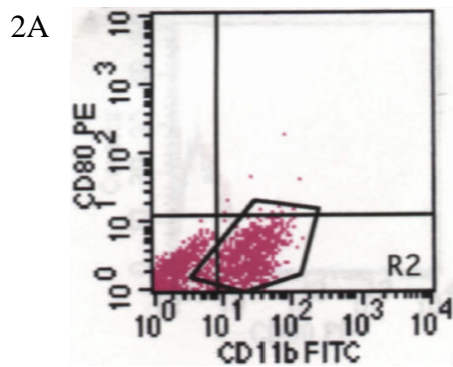

2A

3A

2B

3B

Fig. 2 CD11b$^+$ cells were slightly CD86 positive. A. Single cells were stained with CD11b-FITC and CD86-PE. B. CD11b$^+$ cells were gated and analyzed the CD86 expression. The blue curve represents negative control and the red curve stands for CD86 expression among CD11b$^+$ cells.
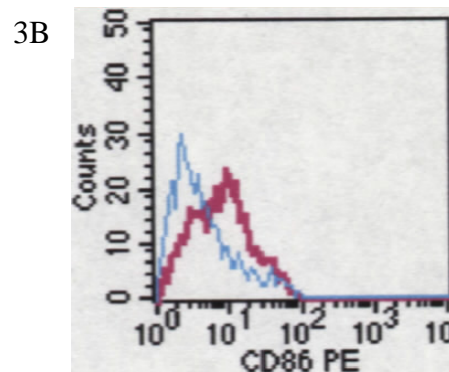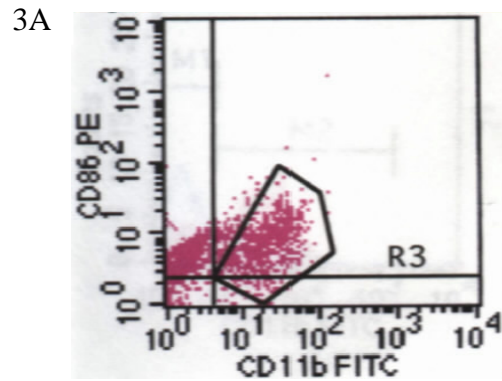
Fig. 3 CD11b$^+$ cells were entirely CD80 negative. A. Single cells were stained with CD11b-FITC and CD80-PE. B. CD11b$^+$ cells were gated and analyzed the CD80 expression. The blue curve represents negative control and the red curve stands for CD80 expression among CD11b$^+$ cells. All CD11b+ cells didn't express CD80 on the surface.

It has been demonstrated that there was high level of the CD30 in human decidual cells. (Placenta. 22:309,2001; Am. J. Pathol. 145: 276, 1994) This implicated that there should be corresponding CD30 ligand (CD30L, CD153) adjacent to CD30. In addition, unpublished data supplied by co-laboratory indicated that CD86 expression was down regulated while CD30-CD30L

To summarize, we found that almost HLA-DR$^+$ cells have CD11b and CD30L expression on the cell surface. It means that there are HLA-DR and CD 30L co-expression at the same time on decidual macrophages. It's a new finding in this field. Besides, these cells were CD80$^-$ CD86^{low}.

It has been suggested "suppressor macrophages" maybe one of the tolerance

mechanisms of the fetal allograft. Here, we provide new insight to the possible role of decidual macrophages in normal pregnancy. Based on these references and findings, we suggest that the stromal cells maybe the source of CD30 in decidua. Upon interaction
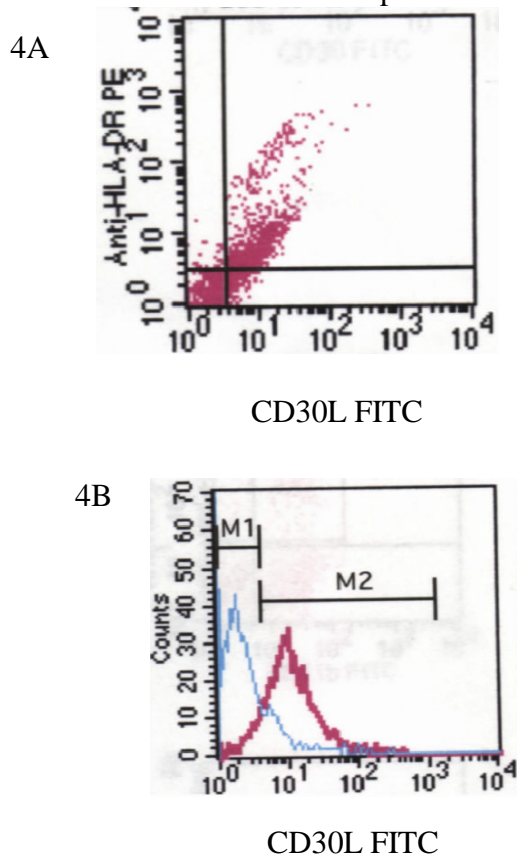
4A

CD30L FITC

4B

CD30L FITC

Fig. 4 HLA-DR$^+$ cells were CD30L$^+$ and the CD30L was medium expression. A. Live cells were gated and analyzed surface CD30L-FITC (FL1) and HLA-DR PE (FL2) expression. B. HLA-DR$^+$ cells were gated for further detection of CD30L expression.

between CD30 on stromal cells and CD30L on decidual macrophages, the antigen presentation ability of decidual macrophages was diminished by down regulation of co-stimulator (CD80/CD86) expression. Thus, T cells infiltrated in deciduas become anergy and tolerant to the fetus. But what's the exact mechanism that decidual macrophages utilized remains unclear and need further investigation.

四、計畫成果自評

In the current study, we demonstrated at the first time that decidual macrophages have CD30L on the surface. The unique molecule may provide some signaling while ligating to the corresponding receptor, CD30. Although we have proposed one possibility to explain the phenomenon observed here, it needs more experiments and solid findings to support our suggestion. At the same time, we have been trying to isolate these decidual macrophages for further study with limited success. Currently we have finished the study in 24 normal pregnancies and 12 anembryonic pregnancies. To clarify the sophisticated network in deciduas in normal and abnormal pregnancies might answer certain important issues of the immunlogical functions of those macro- phages. And it will be benefitial to patients with reproductive disorders.

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