Trials of making and detecting human monoclonal antibodies directed against villous trophoblast

T. Fujino, H. Shiokawa, K. Shiraogawa, H. Otsuka, K. Miwa and Y. Nagata
Dept. Obstet. & Gynec., Faculty of Medicine, Kagoshima Univ., Kagoshima 890, Japan.

The role of immune response in the establishment and maintenance of normal pregnancy is still unclear. Recent reports suggest that it seems to be important to know maternal immune responses to trophoblast. Whether or not maternal antibodies are generated against trophoblast during pregnancy is not yet determined. As the first step for elucidating maternal immune responses to trophoblast, in this study we tried to make and detect human monoclonal antibodies directed against villous trophoblast using Epstein-Barr virus (EBV).

The cell line B 95-8 was used as a source of infectious virus. The virus particles were harvested and concentrated. Peripheral blood mononuclear cells from normal puerperal women (post partum day 1) were infected with concentrated EBV and incubated in alpha-medium supplemented with 10% fetal calf serum and 1% phytohemagglutinin and without feeder layer.

Four weeks after infection with EBV immunoglobulin (Ig) levels were measured in the supernatant medium from each well of cultured B lymphocytes. In most wells infected B cells produced much amount of Ig. The average level was about 1 μg/ml for IgG and about 100 μg/ml for IgM.

Reactivity of the culture supernatants from Ig producing wells to placental tissues of their own was assessed by immunocytochemistry. Term placenta was obtained soon after delivery and pieces of chorionic villous tissues were dissected from a central cotyledon. They were snap frozen in liquid nitrogen and cryostat sections (7um) were cut. The indirect immunoperoxidase technique which was used to detect human monoclonal antibodies directed against placenta is shown in Fig.1.

As endogenous antibodies are often bound to placenta, the second layer antibody, goat anti-human Ig peroxidase conjugate may react to them resulting in non-specific staining. To prevent this undesirable reaction the second layer antibody was used in such a concentration that it did not react to placenta sections without the first layer, that is, culture supernatant. Negative...
Cryostat sections of chorionic villous tissue fixed in acetone

\[ \text{Culture supernatant} \]

Wash in three 5-minute change of PBS

\[ \text{Goat anti-human Ig } \text{ properly diluted} \]

Wash in three 5-minute change of PBS

Colour reaction with DAB

Wash in three 5-minute change of PBS

Nuclear staining

Wash in water

Dehydration and mount in DPX

Fig 1. IMMUNOPEROXIDASE TECHNIQUE
controls were employed using culture medium.

By this immunoperoxidase technique we found that supernatants from some wells had IgM which reacted to villous stroma, and syncytiotrophoblast as well suggesting that normal pregnant women recognize their syncytiotrophoblast and make antibody against it. But, as each well had four to ten B cell clones, cloning should be done successfully to obtain human monoclonal antibody directed against syncytiotrophoblast.

Further investigations could be done about maternal immune responses to trophoblast by this technique using EBV.

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