Pregnant sera induce selective granulocytic differentiation of HL60 cells.

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Materials and methods
Pregnant and non pregnant sera were collected from subjects who visited Nihon University Itabashi Hospital since May to September 1989. HL60 cells were kindly provided by SRL Co.

The cells were cultured in 25 cm² culture flasks with RPMI1640 media, supplemented with 10% fetal calf serum (GIBCO). Cultures were incubated at 37°C in a humidified atmosphere of 5%CO₂ and 95% air. The medium was changed every 4 days during maintenance. Another series, pooled pregnant sera or pooled nonpregnant sera were added to these culture instead of FCS.

Cell numbers were counted everyday for 1 week, evaluating culture viability with trypan blue staining. Then cells were examined morphologically with Wright-Giemsa stain. On the 6th day of culture, cells were exposed to flow cytometric analysis using FITC conjugated anti OKM1(CD11b) and anti HLA-DR monoclonal antibodies (Ortho Diagnostic Co.). From 1X 10⁷ cells, total RNA was extracted by RNA zol system (Sinna Bio) at 4.7 days of culture. 1, 3, 10μg of RNA samples were blotted on Hibond-N membrane (Amersham) with Minifold II slot blot apparatus (S&S). Hybridization was carried out with human c-myc 3rd exon DNA probe (1.5 kb).

Results
1. Cell growth: No difference was noted in the cell growth curves between FCS, PHS (pooled human sera) and PPS (pooled pregnant sera) as shown in fig 1.
2. Morphological differentiation;
Up to 7 days of culture, about 10% of the cells differentiated into granulocyte/monocyte like cells. However no difference was noted in differentiation rates of cells cultured in FCS, PHS, and PPS (table 1).

3. Flow cytometric analysis;
Significant increases (P<0.05) of OKM1 positive cells with pregnant sera collected from 2nd and 3rd trimester pregnancies were observed while the increase of HLA-DR positive cells was not significant. (table 2)
4. c-myc expression: Pregnant sera, collected from 1st, 2nd and 3rd trimester pregnancies suppressed c-myc expression to 30% of FCS control both at 4th and 7th day of culture, while suppression was not remarkable in cultures with PHS. (fig. 2)

5. Levels of G-CSF in sera employed in this experiment were examined by ELSA. No remarkable increase of G-CSF was noted in sera collected from pregnant subjects. (under detectable levels; 30 pg/ml)

Discussion

In order to study events related to human granulocytic/monocytic differentiation in vitro, we have utilized the HL60 human promyelocytic leukemia cell line. This cell line is capable of continuous proliferation in vitro as transformed promyelocytes and the reinitiation of the terminal differentiation process with natural and synthetic inducers.

In this study we failed to note significant difference in pregnant and non pregnant sera in growth rate or differentiation assessed by Wright Giemsa morphology. We therefore attempted to detect and quantitate the differentiation induction by flow cytometry using well known surface markers, and found selective increases of OKM1 positive cells by addition of pregnant sera. Mature granulocytes are OKM1 positive and HLA-DR negative while mature monocytes are OKM1, HLA-DR double positive. Thus our data suggest selective induction or commitment of HL60 into a granulocyte lineage by pregnant sera.

The exact molecular mechanism of this commitment is unknown and several candidate substances in pregnant sera can be attributed to this process. One of the most possible candidates is G-CSF. However we failed to note remarkable increase of G-CSF in sera collected from pregnant subjects.

Not only cytokines but many pregnancy associated substances such as steroid and peptide hormones, fetoplacental antigens etc. must be examined.

Alternatively, an entirely unique pregnancy associated factor(s) could induce granulocytic differentiation of HL60 cells in vitro and play possible
important roles in vivo, and account for their functional increase in pregnancy. OKM1 induction activity of pregnant sera was not remarkable in 1st trimester PPS. However it suppressed c-myc expression in HL60 cells. Generally it is accepted that c-myc expression correlates with cell proliferation and it is suppressed with cell differentiation or cell sedation. We have as yet no adequate explanation for this discrepancy. We have reported on the direct suppressive effect of pregnant sera on c-myc expression in PHA activated lymphocytes together with suppression on IL-2/IL-2R autocrine stimulation. (Hayakawa, 1988) Possibly factors in pregnant sera responsible for c-myc suppression and differentiation induction are distinct and these phenomena are independent. Further investigations are required to better understand of these problems.

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Reference