PRENATAL HLA-DETERMINATION.

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The present paper comprises the methods used for prenatal HLA-determination in a number of cases. When live cells are available, through a villous biopsy, amniocenthesis, or directly from an aborted fetus, a cytotoxic microtechnique is applied on cultured cells (cell culture: L.O. Vejerslev et al., this issue). After spontaneous or induced abortion a microabsorption method with the fetal tissue may be used.

For the cytotoxic microtechnique special reagents and selected complement are needed.

HLA antisera: Reagents are obtained through the laboratory screening programme, and tested on B- and T-lymphocytes at room temperature and at 37°C (Hansen & Gundolf, 1981). After throughout testing on peripheral lymphocytes, the sera are tested for possible use on cultured cells. Sera which act operationally monospecific in routine HLA-typing very often show unexpected positive reactions with cultured cells. The cultured cells seem to be more vulnerable, and thus more susceptible to weak antibodies and/or crossreactions which would not effect normal peripheral lymphocyte suspensions. Some reagents operationally monospecific in routine typing give strong unspecific positive reactions with all cultured cell populations. So far this phenomenon remains unexplained.

Complement: Most normal rabbits have antibodies toxic to human cells. To assure non-toxicity of the rabbit complement it is necessary to test the toxicity of the serum of each rabbit separately. Both B- and T-lymphocytes, freshly prepared as well as frozen and thawed, are used in three non-toxic media. Rabbit sera, which are absolutely non-toxic to

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all six test cell suspensions, are tested for complement activity by means of HLA antisera with a well known antibody titer. Rabbits with non-toxic serum often have a poor complement activity. As a result 0-2 rabbits out of 10 may pass the test. The complement used is a pool of serum from 4-6 animals. The rabbits are bled for 40 mls of blood about once a month. The serum is separated within two hours, pooled and stored in liquid nitrogen. Once or twice each year one animal in the group is replaced by a new one.

The cytotoxic microtechnique: The method is essentially the same as described for HLA typing of cells from hydatidiform moles (Hansen & Vejerslev, J. Immunol. Meth., in press), and is used when the cells are grown in monolayer cultures for HLA determinations in situ. For the prenatal determinations two variants of the cytotoxic technique have been used: a modification of the two stage NIH-technique and a modification of the Kissmeyer-Nielsen (KN) method (fig. 1). So far 10 cultures of amniotic fluid cells, 22 cases of hydatidiform mole, and 3 cultures from normal villous biopsies have been investigated, furthermore in three cases cultured fetal fibroblasts from an aborted fetus have been typed.

Amniotic fluid cells could be typed readily, though with the best results in the NIH-modification, since they often gave weak results in the KN. Cells cultured from villous biopsies could not survive at room temperature, and the best results were thus obtained in the modified KN-technique. Cultured fetal fibroblasts from the fetuses could be typed in both cytotoxic techniques.

In one case microabsorption of specific HLA antisera with a crude suspension of fetal liver tissue from an aborted fetus was performed. The microabsorption method has been described in details elsewhere (Hansen & Gürtler, 1981 & 1983). In this case 30 µl samples of centrifuged fetal liver was mixed with 50 µl of selected, specific antisera, and incubated for 1 hour at 37°C before the serum was recovered. The ability of the fetal liver to reduce specifically the antibody titer of the HLA antisera was then measured in the routine cyto-

toxic test using lymphocytes from appropriate test donors. The case is described as case c in fig. 2. In this case it was possible to distinguish, by absorption, the crossreacting antigens A2 and A28, because the serumbattery comprised two antisera reacting with A2 and not with A28, and three sera reacting with A2-A28, and the antibody activity was not reduced in the two reagents reacting only with A2. In the same way two reagents containing anti-B5+B53 showed no reduction in antibody activity after absorption, while the antibody was removed in an anti-B35+B5.

Conclusion: A prenatal HLA-determination is possible after the 11th week of gestation with these methods. Investigation of the HLA type of a fetus may be indicated for medical reasons for example for diagnosis of the HLA linked trait, congenital adrenal hyperplasia. In forensic medicine an HLA-determination of a fetus during pregnancy could be indicated for example in cases of rape, in which doubt exists about the paternity of the fetus, as well as in case of abortion following rape or sexual abuse.

References:

Hansen, H.E. & F. Gundolf (1981) Frequency and specificity of HLA-A,B,C,DR antibodies in the sera of pregnant women. The practical outcome of tissue typing reagents through screening. In: 9. Internationale Tagung der Gesellschaft für forensische Blutgruppenkunde, Bern, 1981:10-14.

Hansen, H.E. & H. Gürtler (1981) HLA typing of dead bodies. In: 9. Internationale Tagung der Gesellschaft für forensisische Blutgruppenkunde, Bern, 1981:52-56.

Hansen, H.E. & H. Gürtler (1983) HLA-types of mummified Eskimo bodies from the 15th century. Amer. J. Phys. Anthropol. 61:447-453.

Hansen, H.E. & L.O. Vejerslev: Hydatidiform Mole and HLA-Methods for HLA-A,B,C-determination. J. Immunol. Meth. In press.

Vejerslev, L.O., H.E. Hansen, J.G. Westergaard, & F. Søndergaard: Amniotic fluid cell and chorionic villi culturing for prenatal HLA-determination. This issue.

Fig. 1. Complement dependent cytotoxic microtechnique for prenatal HLA-determination.

Microchambers with 60 wells à 10 µl.

A modified NIH-technique:

No medium in the wells.
2 µl of Hanks' solution+20% FCS/well.
Wells covered by paraffine oil.
+ **2 µl** HLA antiserum/well.

30 minutes at room temperature.

+ 4 µl **rabbit** complement/well.

60 minutes at room temperature.

+ 2 µl trypanblue solution (0.1%)/well.
20 minutes at room temperature.

A modified KN-technique:

No medium in the wells.
6 µl of Hanks' solution+20% FCS/well.
Hanks' solution **replaced** by **4 µl** HLA antiserum/well.
Wells covered by paraffine oil.

At once:

2 µl **human** + 2 µl **rabbit** complement/well.

30 minutes at 37°C.

+ 2 µl trypanblue solution(0.1%)/well.
20-30 minutes at room temperature.

READING.

Number of dead, stained cells in each well counted by means of an inverted microscope according to the scale:
0-5% : -, 50% : ++, 75% : +++, 100% : ++++

Fig. 2. Examples of prenatal HLA determination.

a) Cultured cells from amniotic fluid, sampled at the 16th week of gestation.

Mother : HLA-A3,A11;B15,B40;Cw2
Father : HLA-A2,A11;B21,B44;Cw-
Fetus : HLA-A11 ;B21,B40;Cw2
Cord blood, at delivery : HLA-A11 ;B21,B40;Cw2

Index = 310.0

b) Cultured cells from villous biopsies taken in or before the 11th week of gestation have given negative reactions in the cytotoxic technique.

Cultured cells from a villous biopsy taken at the 15th week of gestation.

Mother : HLA-A1,A11;B7,B40;Cw3
Father : HLA-A1,A31;B7,B40;Cw3
Culture: HLA-A1,A31;B7,B40(Cw3 not tested)

Index : 60.2

c) Aborted fetus, 16th week of gestation.
Microabsorption with fetal liver tissue: HLA-A3,A28;B16,B35 Cw4: only one serum available, weak absorption.
Cytotoxic test on cultured fetal fibroblasts: HLA-A3,A28;B35,B16;Cw4.(Cw4: only one serum available).

Combined results of case:

Mother: HLA-A3, ;B7,B35;Cw4
Man : HLA-A2,A28;B5,B39
Fetus : HLA-A3,A28;B35,B16(B38/B39);(Cw4)

Index = 188.3