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Immunoteratogenesis – a New Field of Investigation

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Abstract: The normal human serum (NHS) exerts an embryotoxic effect on chicken embryos. The effector of its embryotoxic action is complement. Sera deficient in some components of the complement system (C1q, C3, C9 or B) lose their embryotoxic capability.

Decrease and even disappearance of NHS embryotoxicity can be achieved by incubation with chick embryonic tissue. The inactivating effect is stage dependent. Similarly, the human embryonic tissue is capable of saturating embryotoxic factor from NHS. We demonstrated that the embryotoxic factor – human complement – may be bound to the human target. This is crucial for a new investigation of possible pathological action of complement during early stages of human pregnancy.

Thsi hypothesis is supported by recent studies about function of the complement and its regulatory proteins in human reproduction.

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A considerable and constant part of human reproductive outcome is represented by spontaneous abortions, sometimes even recurrent. Increased incidence of abortions was recently demonstrated in areas with high polluted environment (e.g. Northern Bohemia). In physiologically normal pregnancy the embryo is seldom rejected although it represents a subject immunologically different from and foreign to the mother organism. Our present study is focused on the possibility that at least in part the abnormal pregnancy outcome may be caused by insufficient defense mechanism of the conceptus against maternal immune graft rejection mechanism – the so called immunoteratogenesis.

It was demonstrated by Jelínek et al. (1980) that normal human serum (NHS) exerts a distinct deleterious effect when injected intraamniotically to 4-day-old chick embryos. NHS produced a typical malformation syndrom which comprised coelosomia (defect of the anterior body wall), orofacial, brain and heart abnormalities. Accompanied with amniotic contraction, it was called the “strait-jacket syndrom”.

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The effect of sera exhibited a considerable individual variation and numerous experiments performed at that time resulted in hypotesis that the embrytoxic factor within human sera is the complement system. The direct proof was remained, however, lacking.

Aim of the Study

The aim of the present study was:

1. To verify the hypothesis that the effector of embryotoxic action is hemolytic complement, and
2. To support the idea that complement may play a role in affecting human conceptus.

Design of the Study

As an experimental object we used chick embryos on incubation day 4 (staged 20–24 following Hamburger and Hamilton 1951). Intraamniotic injections of 3 ul of NHS were performed by a microcanule with obliquely ground tip under the binocular preparation microscope. Each experimental group consisted of about 30 specimens. Th windowed eggs were closed with glass slides allowing easy control, sealed with paraffin and reincubated for 5 consecutive days. After everyday checking the embryos were harvested on incubation day 9, inspected and dissected under the microscope. An index of embryotoxicity Es was calculated for each group, comprising survival times and severity of malformations detected.

To sustain the first hypothesis we used sera deficient in some components of the complement system. We applied C1q-deficient serum to study classical C′-pathway action on embryo and B-deficient serum to study the alternative pathway. C3-deficient and C9-deficient sera were studied because of the importance of C3 and C9 component in C′-activation shared by the both pathways. It was also observed, whether the membrane attack complex (including C9) or anafylatoxins (C3a, C5a) may cause the malformations. All these commercially obtained deficient sera were found to be significantly less embryotoxic to the chick embryo than the standard NHS (Fig. 1).

We also demonstrated the potential of chick embryonic tissue to saturate the embryotoxic factor (EF) from NHS. We incubated NHS in 37 °C for 60 min with mechanically desintegrated chick embryonic tissues and the resulting product was than injected to chick embryos. Embryotoxicity of NHS appeared significantly decreased in all cases (Fig. 2).

To make sure that the saturation effect was not caused by the species different tissue only, in other words to demonstrate that the embryotoxic factor may be similarly bound to the human target, we carried out a similarly designed pilot study with human embryonic tisue. A convincing decrease of embryotoxicity of serum incubated with human embryonic tissue (obtained by 5–7 week-old arteficially aborted specimens) was observed compared with standard NHS This suggests that human conceptus may be endangered by directly acting complement similarly as the chick embryo.

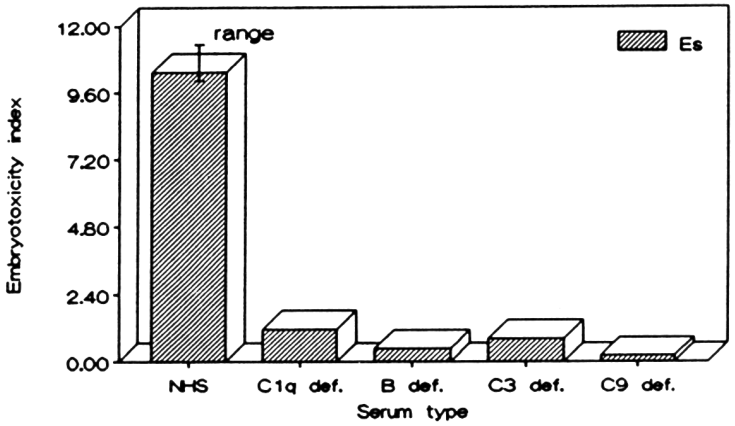

Fig. 1. NHS – chick embryo. Effects of complement deficient sera.

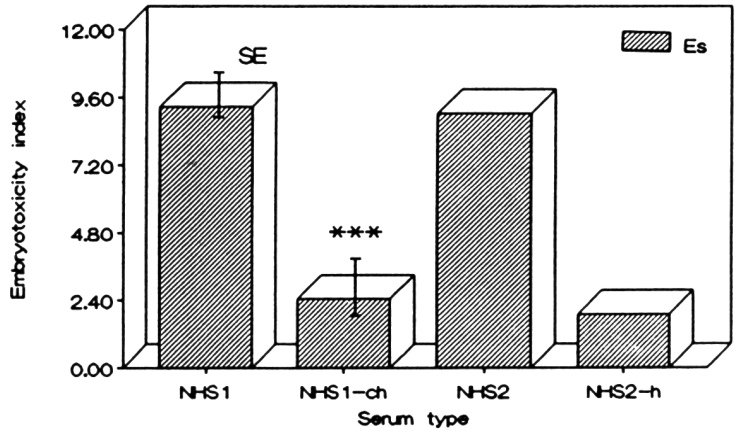

Fig. 2. NHS – chick embryo. Effect of incubation with target tissues.

Conclusions

C′ components-deficient sera and NHS incubated with target embryonic tissues has been shown to decrease embryotoxic activity of the reference standard NHS. This allows us to make following conclusions:

1. EF of NHS is the complement system which may be activated by both classical and alternative pathways.
2. Decrease and even disapearance of NHS embryotoxicity can be achieved by inubation with chick embryonic tissue. The inactivating effect appears stage dependent. The younger the source of embryonic material, the weaker the saturating effect which coresponds to the sensitive period for the complement embryotoxicity.
3. Similarly, the human embryonic tissue is capable of saturating EF from NHS. This piece of knowledge is crucial for a new investigation of possible pathological action of complement during early stages of human pregnancy.

Recent Studies

This hypothesis is supported by recent studies about function of the complement and its regulatory proteins in human reproduction.

The production of C′ by the endometrial cells and its hormonal regulation was observed. (Hasty et al. 1994)

Complement regulatory proteins (MCP, DAF, CD59) that inhibit alternative and classical pathway of C′-activation are strongly expressed by the trofoblast and amnial epithelium already in early stages of human development. The human embryo seems to be protected by CR-proteins from the embryotoxic effect of complement (Katz et al. 1995).

A part of recurrent spontaneous abortions (RSA) is characterized by decrease of CR-proteins in trofoblast (Cunningham et al. 1995).

The serum C′-activity was quantified so as to define the dynamics of C′-activation in early pregnancy loss. A part of RSA demonstrated activation of C′ by the alternative pathway as early as 7th week with the progressive decline in C′-activity until abortion was clinically completed (Tichenor et al. 1995).

Conclusion

Immunoteratogenesis – a newly described mechanism of teratogenesis was documented on chick embryos treated with heterologous vertebrate sera. However, our study also suggests a potential involvement of teratogenic action of complement in some pregnancy pathologies, namely in recurrent spontaneous abortions.

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