5. V. B. Sboichakov, A. M. Korolyuk, V. N. Verbov, *et al.*, *Zh. Mikrobiol.*, № 7, 83-86 (1986).
6. N. V. Sorokina, E. M. Gavrilova, A. M. Egorov, and N. A. Koltovaya, *Ibid.*, № 12, 68-72.
7. N. F. Timchenko, *Data on the Microbiology of the Agent of Far-Eastern Scarlatiniform Fever (Human Pseudotuberculosis)* [in Russian], Thesis of Cand. Med. Sci., Vladivostok (1972).
8. G. F. Shpilyuk, T. N. Goloveshkina, I. K. Volodina, *et al.*, *Zh. Mikrobiol.*, № 6, 92-93 (1993).
9. M. Ynone, H. Nakashima, T. Yshida, and M. Tsubokura, *Zentralbl. Bakteriol. Mikrobiol. Hyd.*, **186**, 504-511 (1988).

Antibodies to *Bacillus megaterium H.* Glycoprotein in Pregnant Women with a Pathological Course of Pregnancy

A. I. Autenshlyus, O. V. Ivanova, V. V. Gauzer, and V. A. Semernikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 8, pp. 203-205, August, 1995
Original article submitted September 30, 1994

The humoral immune response to *Bac. megaterium H.* glycoprotein was studied in women with normal and abnormal pregnancies. The level of antibodies to bacterial glycoprotein was elevated in women with chronic foci of infection during the second and third trimesters. Elevated levels of antibodies to *Bac. megaterium H.* reflect the presence of pathological changes in the fetus during intrauterine development and may be used as prognostic criteria of intrauterine disease.

Key Words: *antibodies; glycoprotein; pregnant women*

The levels of antibodies to *Bac. megaterium H.* are elevated in patients with tumors of various localization, due to the presence of common antigenic determinants in tumor cells and in bacterial glycoprotein [4]. Fetal and tumor cells are known to possess common antigens, and fetal antigens induce an immune response in cancer patients [3,5,7]. For this reason we decided to study the humoral immune response to *Bac. megaterium H.* in pregnant women and analyze the data in comparison with the health status of the newborns.

MATERIALS AND METHODS

Sera of 148 pregnant women divided into 3 groups depending on the health status of their newborns were tested. The main criterion of infant health was the status of the central nervous system, the involvement of which is the most frequent pathology during the neonatal period and infancy, reflecting intrauterine disease of the fetus. Group 1 consisted of 12 pregnant women whose infants were considered to be healthy, group 2 was made up of 71 women who gave birth to children with manifest disorders of the central nervous system which persisted in one form or another after the age of 2, and group 3 consisted of 65 women whose infants presented with less pronounced abnormalities of the CNS which disappeared by the age of 2 as a result of therapy. All groups were matched for age and obstetric history.

Glycoprotein with a molecular weight of 65 to 70 kD isolated from *Bac. megaterium H.* was used as antigen. Antibodies in the sera of pregnant women were detected by enzyme immunoassay (EIA).

Glycoprotein solution (100 μl) in carbonate-bicarbonate buffer, pH 9.0±0.2 was pipetted into plates in a concentration of 100 μg/ml and incu-

Institute of Medical and Biological Cybernetics, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk (Presented by V. V. Lyakhovich, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0008-0846$12.50

bated for 16-18 h at 4°C. The contents of the plate were discarded by shaking and the plate was washed twice with buffered normal saline with an added detergent (Tween-20), after which 200 μl of 2% bovine serum albumin were added to each well and the plate was incubated for 1 h at 37°C. The contents of the plate were removed by shaking, and 100 μl of the test sera diluted 1:50 were pipetted into the wells. The sera were incubated for 1 h at 37°C, after which the plates were washed three times. 100 μl of antibodies to human immunoglobulins labeled with horseradish peroxidase (manufactured by the Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences) were introduced into each well and incubated for 1 h at 37°C, after which the plates were washed three times. Then 100 μl of substrate mixture were placed in each well, the mixture being prepared *ex tempore* and consisting of citrate buffer, pH 4.7, methanol, ortho-phenylenediamine, and concentrated hydrogen peroxide. The plate was put in a place shielded from the light until color appeared in the wells. The reaction was arrested by the addition of 100 μl of 1 M hydrochloric acid. The results of analysis were assessed photometrically. Serum of a healthy woman with a normal pregnancy was the negative control. The results were processed using the $K=Do/Dc$ coefficient, where Do is the mean optical density of the test serum and Dc the mean optical density of the negative control serum. K values higher than 1 were considered positive. The results were statistically processed using Student's test.

RESULTS

Group 1 pregnant women possess a baseline level of antibodies, as shown by EIA. This control level is caused by increased penetration of antigenic substances (glycoproteins) of the embryo into the maternal bloodstream through an amorphous layer of chorionic trophoblast starting from gestation weeks 4-5, when the fetal circulation appears. The maximal level of high-polymeric substances (immunologically active) occurs during early pregnancy, at weeks 5-6; by weeks 11-12 their content is appreciably decreased and it continues to diminish progressively up to the end of normal gestation [2,6].

However, elevated levels of antibodies to bacterial glycoprotein were detected in 60% of the women in group 1 during the first trimester and in 33.3% in the second trimester. This may have been due to somatic diseases and extragenital foci of infection, as well as to threatened abortions in early pregnancy.

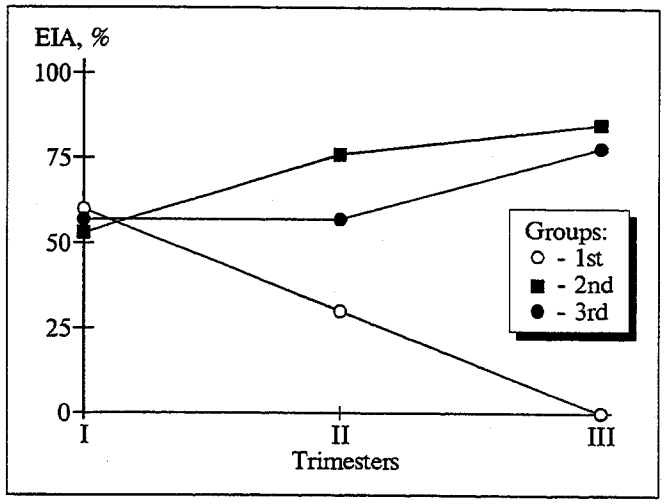

Fig. 1. Incidence of positive results of EIA with *Bac. megaterium H.* antigen during various stages of pregnancy.

The incidence of high levels of antibodies to *Bac. megaterium H.* in group 2 was appreciably higher than in group 1: 71.8%, the distribution of positive results at various times of gestation markedly differing from the curve for group 1 (Fig. 1). In group 1 the highest percentage of positive EIA results was observed in the first trimester; in contrast, in group 2 the number of positive results increased as pregnancy progressed, the maximal level, 82.6%, being observed during the third trimester.

In group 3 high levels of antibodies to bacterial glycoprotein were detected in 61.5% of cases. Analysis of the incidence of positive results of EIA at various stages of pregnancy showed it to be similar to that in group 2: the number of positive results was highest (76.2%) in the third trimester, in comparison with 57.1 and 56.7% in the first and second trimesters, respectively.

Hence, the results suggest that the incidence of positive results of EIA with bacterial glycoprotein was virtually the same in all groups during the first trimester, evidently due to the absence of a placental barrier in the early period of gestation and to massive release of embryonal antigens into the maternal circulation. Structural and functional maturation of the placenta leads to a lesser penetration of embryonal proteins into the maternal organism during normal pregnancy in healthy women. In the event of a chronic focus of infection, particularly of a urogenital localization, of a pathological course of pregnancy, or a viral infection during pregnancy the placental complex loses its protective potential and becomes easily penetrated by fetal antigens throughout gestation. This is clearly demonstrated by the results in groups 2 and 3, in which 12 and 9% of women, respectively, were healthy, with extragenital foci of in-

TABLE 1. K Values in Groups of Women Examined during Different Trimesters of Pregnancy ($M \pm m$)

Group	Trimester		
	I	II	III
1	0.98±0.07 $p_{1,3}>0.05$	0.99±0.05 $p_{1,3}<0.01$	0.87±0.04 $p_{1,3}<0.001$
2	1.07±0.06 $p_{1,2}>0.05$	1.33±0.08 $p_{1,2}<0.01$	1.28±0.05 $p_{1,2}<0.001$
3	1.09±0.06 $p_{2,3}>0.05$	1.17±0.05 $p_{2,3}>0.05$	1.42±0.08 $p_{2,3}>0.05$

fection detected in 63 and 51% of cases, respectively, and foci of urogenital localization in 45 and 34% of cases. Pregnancy ran a pathological course in virtually 90% of the women in groups 2 and 3, this explaining such a high incidence of elevated levels of antibodies to bacterial glycoprotein.

Analysis of the mean K values in the three groups showed reliable ($p<0.001$) differences between the EIA results in group 1 (0.95±0.03) and in groups 2 and 3, in which the mean values for the groups were the same (1.24±0.04). The data on the mean K values for various gestation periods are presented in Table 1, demonstrating that the results did not differ reliably in any of the groups during the first trimester, while in the second trimester reliable differences ($p<0.01$) in K values were observed between groups 1 and 2 and 1 and 3, but not between groups 2 and 3. As pregnancy progressed, the reliability of the differences between groups 1 and 2 and 1 and 3 increased to $p<0.001$, but the differences between groups 2 and 3 were still unreliable, the mean K values for group 3 in the third trimester being higher than in group 2, although the difference was unreliable. The higher K values in group 3 women during the third trimester may have been due to the higher (double) incidence of exacerbations of chronic infections. This apparently caused increased permeability of the histohematic barriers and resulted in an enhanced release of fetal antigens, which by this time have attained a high level of immunogenic activity into the maternal circulation.

Hence, our findings on the humoral immune response to *Bac. megaterium H.* glycoprotein indicate that this bacterial glycoprotein belonging to tumor-associated antigens possesses common antigenic determinants with embryonal proteins, this appreciably broadening the scope of its use for diagnostic and prognostic purposes.

REFERENCES

1. A. I. Autenshlyus, V. A. Semernikov, S. V. Khutornoi, *et al., Byull. Eksp. Biol. Med.*, **111**, № 5, 525-528 (1991).
2. *Problems of Adaptation in the "Mother-Fetus" System (Immunological Aspects of Pregnancy)* [in Russian], Novosibirsk (1973), p. 155.
3. V. I. Govallo, *Reproduction Immunology* [in Russian], Moscow (1987), p. 304.
4. D. G. Zatula and V. A. Semernikov, *Immunology of Cross-Reacting Antigens of Microorganisms and Blastoma Cells* [in Russian], Kiev (1986), p. 222.
5. A. Bernardini, N. Corbino, P. G. Bapisarda, *et al., Microbiologica,* **5**, 383-388 (1982).
6. I. J. Jacobs, T. N. Fay, J. Yovich, *et al., Hum. Reprod.*, **5**, № 1, 116-122 (1990).
7. G. Pasternak, B. Schlott, J. Reinhofer, *et al., J. Nat. Cancer Inst.*, **69**, 997-1004 (1982).