Immunological reactivity of multiple sclerosis patients during pregnancy and postpartum period

Barac-Latas, Vesna; Morović, Miro; Sepčić, Juraj; Matejčić, Nikola; Rukavina, Daniel

Source / Izvornik: **Acta Facultatis Medicae Fluminensis, 1994, 19, 151 - 158**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:622414

UDK: 612.652: 616.832 - 004.2: 618.4: 618.6
Acta Fac med Flum 1994;19(2):151-158

VESNA BARAC-LATAS[1]
MIRO MOROVIĆ[2]
JURAJ SEPČIĆ[3]
NIKOLA MATEJČIĆ[4]
DANIEL RUKAVINA[1]

IMMUNOLOGICAL REACTIVITY OF MULTIPLE SCLEROSIS PATIENTS DURING PREGNANCY AND POSTPARTUM PERIOD

Departments of
[1] Physiology and Immunology,
[3] Neurology,
[4] Gynaecology,
Faculty of Medicine, University of Rijeka
and
[2] Department of Infectology, General Hospital in Zadar, Croatia

ORIGINAL SCIENTIFIC PAPER

Key words:
pregnancy, puerperium, multiple sclerosis, immunologly

Accepted: 1992-11-25

SUMMARY *Immune response in ten MS patients was studied during pregnancy and puerperal period. Lymphocyte subpopulations in peripheral blood were identified by the rosette forming method. Concentration of immunoglobulins in sera was measured by the radioimmunodiffusion test, and functional reactivity of lymphocytes to polyclonal mitogens by the Hartzman's method. Total and active T lymphocytes showed significantly lower levels during the second and the third trimester of pregnancy. Active T lymphocytes were the lowest in postpartum period. There were no significant changes in percentage of B lymphocytes in pregnant MS patients versus controls. Percentage of total and active T lymphocytes decreased intensively in pregnant MS patients during the relapse. Concentration of immunoglobulins was significantly lower in pregnant MS patients versus healthy nonpregnant women. Concentration of IgG decreased significantly during the third trimester of pregnancy in pregnant MS patients; it was extremely low. Proliferative response to mitogens decreased significantly in pregnant MS patients during the postpartum period in regard to the period of pregnancy. Proliferative response to PHA and ConA measured in pregnant MS patients during the relapse was significantly decreased. It is apparent that gestation is associated with the depression of selective rather than general aspects of cell mediated immunity. Humoral immunity continues to function normally during pregnancy, although opinions about the concentrations of immunoglobulins in the blood of pregnant women are still controversal.*

Multiple sclerosis (MS), a demyelinating disease of the central nervous system, typically results in progressive neurological disability[1]. The etiology of MS remains enigmatic. Although autoimmunity appears to play an important role in the pathogenesis of inflamatory demyelinating lesions[2,3], indirect epidemiological evidence suggests that MS may be induced by an exogenous agent in genetically susceptible subjects[4]. The risk population for MS includes young adults preferentialy women of reproductive age[5].

Virtually all the case reports and reviews published before 1949 concluded that pregnancy affected MS[6,7]. Since than (the 1950's) studies on MS and pregnancy have shown a relative stability of the disease during pregnancy, but a post-partum worsening[8,9,10]. Pregnancy does not appear to be a period of greater risk for relapses, but on the contrary it seems to be a protective event[11]. During pregnancy, humoral immunity, unlike some aspects of cell mediated immunity (CMI), generally continues to function normally[12,13]. It is becoming apparent that gestation is associated with depression of selective, rather than general aspects of CMI. During pregnancy the levels of hormones and other serum factors that may modulate lymphocyte or macrophage synthesis, activation and/or function shift considerably[13]. Many laboratory evidences exist for suppression of the cellular immune response[14,15]. The humoral immune response to exogenous antigens and to the fetal proteins remains intact, while levels of IgG fall only slightly[16]. The aim of this study is an analysis of some aspects of immune response in MS patients during pregnancy and 6 months of the puerperal period.

MATERIALS and METHODS

The clinical course and immunological reactivity were studied in ten clinically definite MS patients during pregnancy and 6 months of postpartal period . Control groups were healthy pregnant and nonpregnant women of the same age. Pregnancy was divided in three trimesters and patients were followed during the postpartum period of six months. Relapse of MS was defined as a worsening or as an occurence of new symptoms or as signs lasting more than 24 hours and less than two months[11]. MS patients and control women were between 20 and 40 years of age.

Immunological analysis

Peripheral blood was obtained by venipuncture and collected in plastic tubes containing heparin. Lymphocytes were separated by Ficol-Hepaque density gradient.

Rosette forming test: lymphocyte subpopulations were detected by method of spontaneus and induced rosette formation (rosette forming cells - RFC) with sheep red blood cells. For E-RFC (total T lymphocytes) and EAC-RFC (B lymphocytes), we followed the method described by Holm et al[17]. Active E-RFC (active T lymphocytes) were determined by our modification[18] of the method described by Smith et al[19]. Active T lymphocytes and B lymphocytes were counted immediately after centrifugation (300 xg), while total T lymphocytes were counted 18 hours later. A lymphocyte with 3 or more sheep erytrocytes attached was considered a rosette-forming lymphocyte.

Immunoglobulins: the concentration (g/l) of immunoglobulins (IgG, IgA, IgM) in sera of patients was measured by standard single radial immunodifusion technique (Meloy plates). Diluted immunoglobulins (IgG 1: 10; IgA 1: 2; IgM was not diluted) were put into RID plates with monospecific antisera toward the corresponding human immunoglobulin and left at room temperature. Diameter of precipitating ring was measured after 50 hours for IgG and IgA and after 80 hours for IgM. Diameters were measured with precision of 0.1 mm RID metre.

Blastic transformation: the functional reactivity of lymphocytes stimulated by polyclonal mitogens (PHA, ConA, PWM) was measured by Hartzman's method[20]. Each test group was cultured in triplicate. The arithmetic mean of triplicate samples was determined, and the results were expressed as the mean number of counts per minute (cpm)+/-SE.

RESULTS

Lymphocyte subpopulations : the analyses of lymphocyte subpopulations showed significantly lower levels of total and active T lymphocytes during the second and the third trimester of pregnancy, as well as during the postpartal period in MS patients (Figure 1). Active T lymphocytes decreased linearly from the first toward the third trimester of pregnancy and postpartal period. Total T lymphocytes decreased linearly during pregnancy and returned to the normal level in postpartal period. There were no significant changes in the percentage of EAC-RFC in pregnant patients versus healthy pregnant and nonpregnant women. In four out of ten pregnant women relapses appeared during the pregnancy. In one during the first, in two during the second

and in the last during the third trimester of pregnancy (black individual spots in Fig.1). The percentage of total and specially of active T lymphocytes decreased intensively in each of the women during the stage of relapse versus mean value of pregnant women. The percentage of active T lymphocytes decreased statistically significantly versus mean value (twice 2%, once 1% and once 11%). These findings are in line with our earlier reports[21] on lymphocytes subpopulations in peripheral blood of MS patients, measured in the active and in the stable phase of the disease. The percentage of active T lymphocytes was significantly lower versus the mean value of the healthy pregnant women.

Immunoglobulins: the level of immunoglobulins was significantly lower in healthy pregnant women and in pregnant MS patients versus healthy nonpregnant women. There were no significant differences between the first two groups. During the third trimester of pregnancy the concentration of the IgG decreased significantly in the pregnant MS patients when compared with the mean value of the control group (nonpregnant healthy women). The value of IgG measured twice in pregnant MS patients with relapses in the third trimester was extremely low (Figure 2- black spots).

Lymphocytes reactivity: reactivity of lymphocytes to PHA and ConA was significantly suppressed in the group of healthy pregnant women, as it is usually seen. There was no significant difference between the reactivity of lymphocytes in pregnant MS patients and that of the healthy women in control group. In two pregnant MS patients with relapses in the second trimester of pregnancy lymphocyte reactivity to the T policlonal mitogens (PHA and ConA) decreased. In two other pregnant MS patients with relapses in the first and the third trimester of pregnancy the proliferative response to the policlonal mitogens was on a normal level.

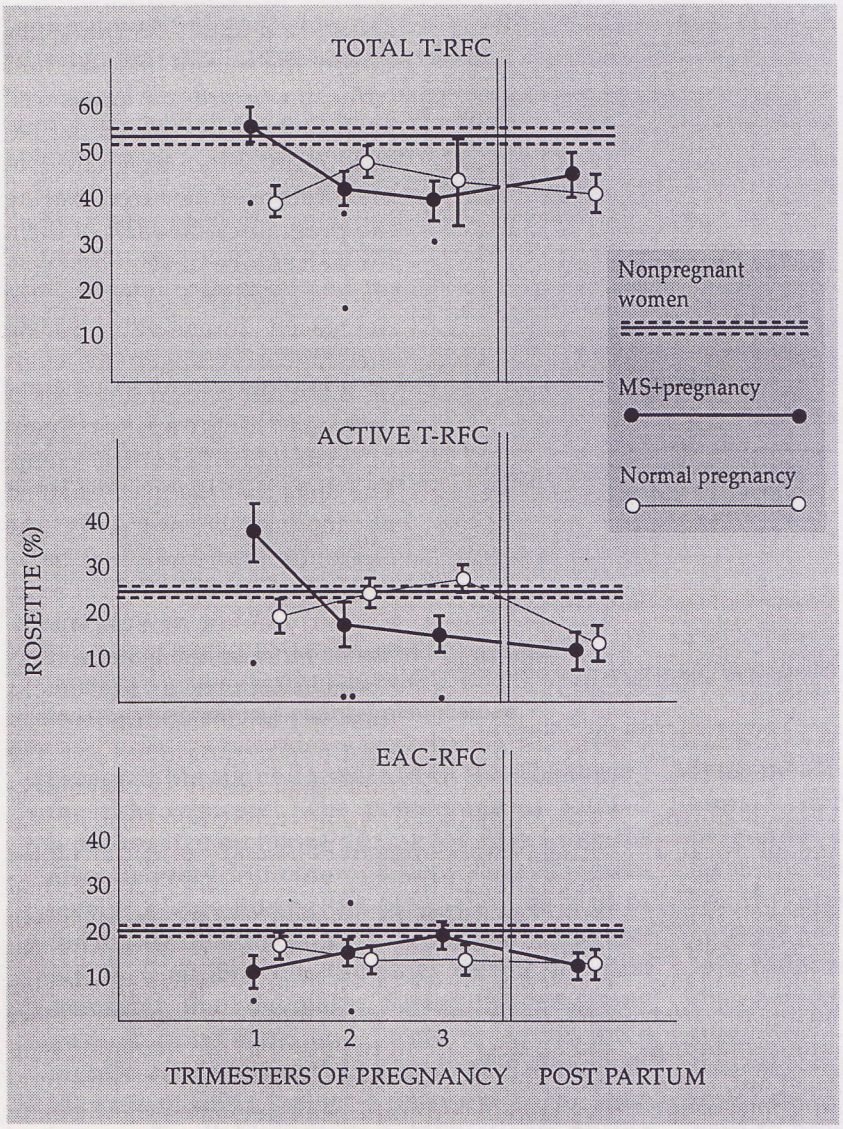

FIGURE 1
Lymphocyte subpopulations in the MS patients during the pregnancy and puerperium period compared with values in healthy

DISCUSSION

Retrospective studies strongly suggest that MS patients enjoy stability during pregnancy, but are at high risk for increased disease activity in the puerperium[8,9,10,11,22]. There is a general agreement among these authors on the magnitude of the postpartum risk. Between 20% and 40% of MS patients will experience a clinical relapse of the disease during three months after delivery. On one hand, MS is the disease of immunological nature and on the other hand, pregnancy is the period of some immunological changes. It has been shown in the literature[23] that the percentage of total T (CD3+) and B (CD20+) lypmhocytes, as well as T lypmhocyte subpopulations (CD4+, CD8+) does not change during the pregnancy,

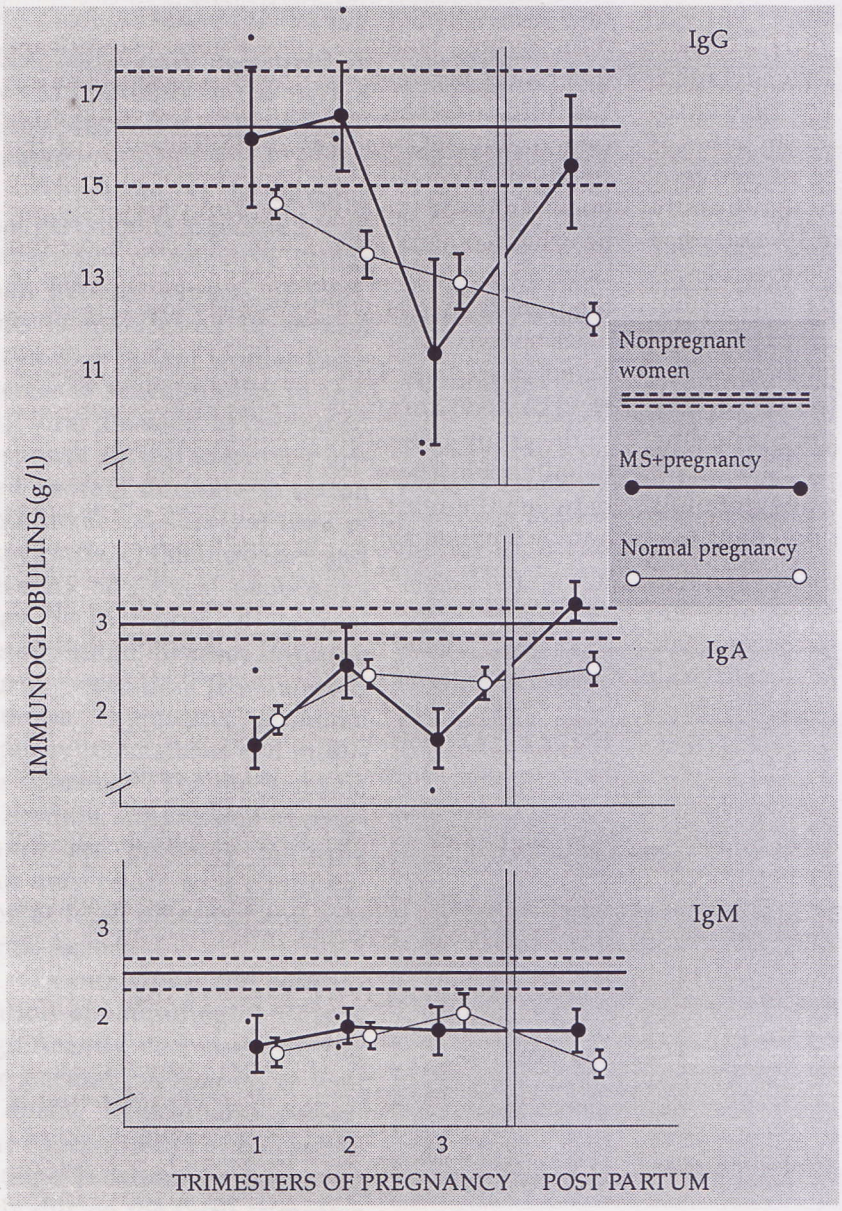

FIGURE 2
Immunoglobulins in the serum of MS patients during pregnancy and puerperium period compared with the values in healthy pregnant and nonpregnant women.

except of the slight decrease of CD4+ cells during the first trimester. The percentage of total T lypmhocytes increases during the first 4 months after delivery. But natural killer (NK) cells intensively change during the pregnancy, as well as during the postpartal period. The percentage of NK/K (Leu 7+) cells increases during the first trimester, than decreases during the remaining part of pregnancy, and increases again for 4 months after delivery[23]. In a study of K. Birk et al[22] the ratio of CD4+ (helper T cells) and CD8+ (suppressor T cells) increases in MS patients during the pregnancy in regard to healthy pregnancy. In these two groups the levels of alpha-fetoprotein, alpha-2-glycoprotein and plasma protein A were the same[22]. Sepčić et al[24], observing the clinical course of MS in pregnant young woman, describe favourable course with suitable levels of T and B lymphocytes, immunoglobulins and C_3 complement. During the relapse (three during the pregnancy) the levels of T and B lymphocytes, specially of active T lypmhocytes, and the reactivity of T lypmhocytes to policlonal mitogens decreased, as well.

In our study the analyses of lypmhocyte subpopulations showed significantly lower levels of total and T lymphocytes during the second and the third trimester of pregnancy, as well as during the postpartal period. In four out of ten pregnant women relapses occured during the pregnancy. In each of them the percentage of total and specially of active T lymphocytes decreased intensively during the stage of relapse. The levels of B lymphocytes did not differ between the groups: pregnant MS patients and control group. The tests that we used enabled us to inspect not only the anatomic (morphologic) background of immunological reactivity (lypmhocyte subpopulations), but also the general functional reactivity of these cells (blastic transformation and levels of immunoglobulins). The functional reactivity of lymphocytes measured by proliferative response of maternal lymphocytes to PHA and ConA during the pregnancy of MS patients, remains on a normal level. The functional reactivity of the same cells in healthy pregnant women was significantly decreased, as it was seen in many other studies before, reflecting an abnormality of function rather than a decrease in the number of circulating lymphocytes[25,26]. Our results show that the proliferative response to mitogens decreases significantly in MS patients during the postpartal period in regard to proliferative response during the period of pregnancy. There are no changes of proliferative response to PWM. Proliferative response to

PHA and ConA measured in two MS patients in the period of relapses was significantly decreased.

Therefore, it is obvious that gestation is associated with depression of selective rather than general aspects of CMI. This is manifested in several ways: a) pregnant women have increased susceptibility to several viral illnesses, b) decreased response to intradermal purified protein derivate injection and increased survival time of skin grafts, c) the incidence and severity of several neoplasms may be increased, d) several autoimmune diseases improve during gestation (rheumatoid arthritis, thyroiditis, systemic lupus erythematosus and multiple sclerosis)[27]. The mechanisms of immune suppression in pregnancy are probably multiple and may relate to a combination of fetal, placental and maternal factors.

In our study the concentration of immunoglobulins is significantly decreased during the normal pregnancy, as well as during the postpartal period. The concentration of IgG in MS patients is extremely decreased only during the third trimester of pregnancy. In the postpartal period the concentration of IgG increases to the normal level. The concentration of IgA is variable, but mostly decreased during the pregnancy as in the case of IgM in both the examined groups (healthy pregnant women and pregnant MS patients). Bisset et al.[28] found an intensive decrease of IgM during the third trimester of pregnancy. It is generaly accepted that humoral immunity continues to function normally during the pregnancy, although the opinions on the concentrations of immunoglobulins in the blood of pregnant women are still controversal.

In several studies the severity and course of EAE were clearly affected by pregnancy or by administration of certain hormones. The ameliorative affect of pregnancy on MS may be related to the suppressive effect of pregnancy on the development of experimental allergic encephalomyelitis (EAE) in laboratory animals[29,30]. In the study of A B Keith[30] none of the pregnant rats aborted or resorbed, all of them giving birth to viable offspring. The phenomenon of delayed onset of disease was observed in pregnant rats. In the study of O Abramsky[29] 80% of animals immunized before the pregnancy developed EAE, but the appearance of the disease was delayed and signs were observed only after full term delivery and never during pregnancy as we excepted. Abramsky et al[31] demonstrated a beneficial effect of fetal alpha-fetoprotein (APF) on the course of EAE in guinea pigs. Arnason and Richman[32] found that oral contraceptives, particulary those high in estrogens, inhibited EAE in rats.

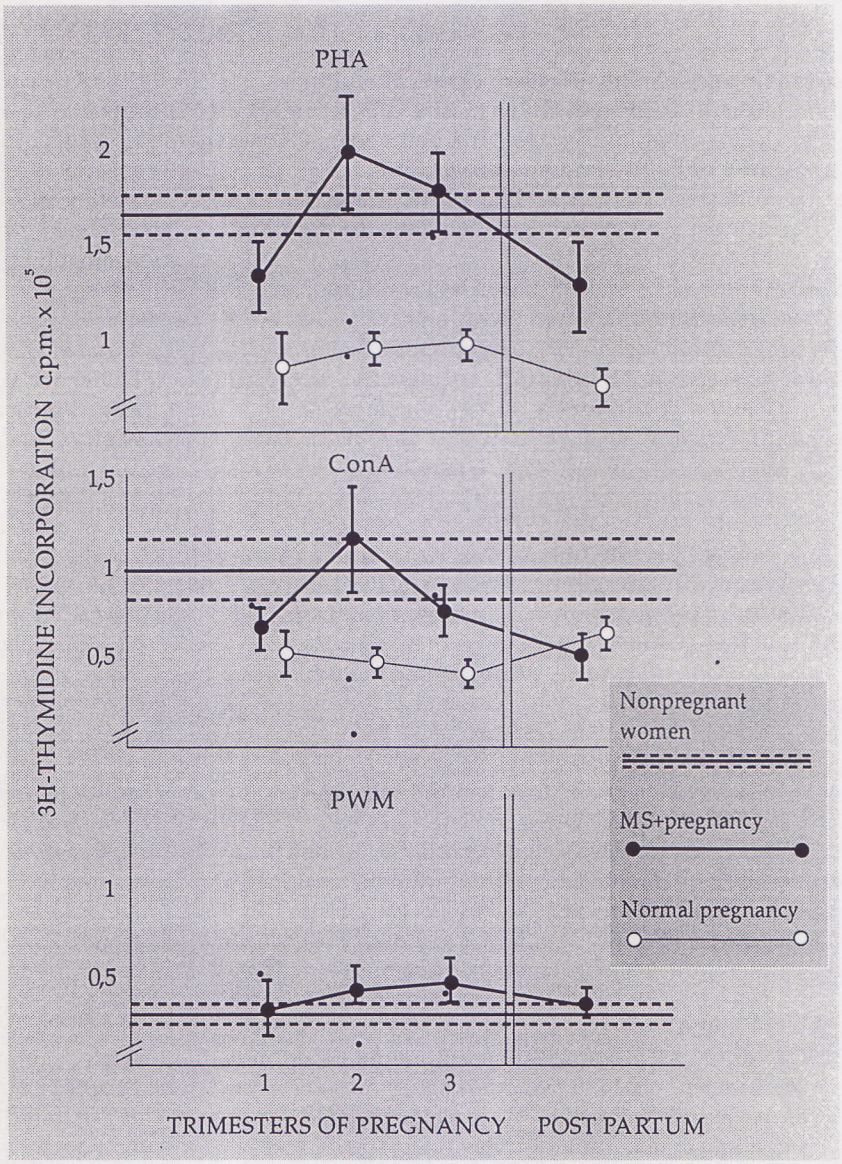

FIGURE 3
Reactivity of lymphocytes to policlonal mitogens in MS patients compared with values of healthy pregnant and nonpregnant women.

REFERENCES

1. *Prineas JW:* The neuropathology of multiple sclerosis, in: "Handbook of Clinical Neurology. Demyelinating Diseases" JC Koetsier, ed., Elsevier Science Publishers, Amsterdam 1985.
2. *Waksman BH, Reynolds WE.* Multiple sclerosis as a disease of immune regulation. Proc Soc Exp Biol Med 1984;175:282-94.
3. *Weiner HL, Hauser SL.* Neuroimmunology. I. Immunoregulation in neurological disease. Ann Neurol 1982;11:437-49.
4. *Waksman B..* Mechanism in multiple sclerosis. Nature 1985;318:104-5.
5. *McArthur JC, Young F.* Multiple sclerosis and pregnancy. From: Goldstein PJ: Neurological Disorders of Pregnancy. Mount Kisco, New York, Futura Publishing Co. 1986;9197:211.
6. *Peckham CH:* Multiple sclerosis complicating pregnancy. NY State J Med 1945;45:618-22.
7. *Douglass LH, Jorgensen CL.* Pregnancy and multiple sclerosis. Am J Obstet Gynecol 1948;55:332-6.
8. *Tillman A.* The effect of pregnancy on multiple sclerosis and its managent. Res Publ Assoc Res Nerv Ment Dis 1950;28:548-82.
9. *Shapira K, Poskanzer D, Newell D, Miller H.* Marriage, pregnancy and multiple sclerosis. Brain 1966;89:419-28.
10. *Korn -Lubetzki I, Kahana E, Cooper G, Abramsky O.* Activity of multiple sclerosis during pregnancy and puerperium. Ann Neurol 1984;16:229-31.
11. *Bernardi S, Grasso MG, Bertollini R, Orzi F, Fieschi C.* The influence of pregnancy on relapses in multiple sclerosis: a cohort study. Acta Neurol Scand 1991;84:403-6.
12. *Adelsberg BR.* Immunology of pregnancy. Mount Sinai Jour of Med 1985;52:5-10.
13. *Weinberg ED.* Pregnancy-associated depression of cell-mediated immunity. Rev Infect Dis 1984;6:814-31.
14. *Wegman T, Gill T.* Immunology of reproduction. New York, Oxfor University Press Inc 1983;pp365-80.
15. *Stahn R, Fabricius HA, Hartleitner W.* Suppression of human T cell colony formation during pregnancy. Nature 1978;276:831-2.
16. *Maroulis GB, Buckley RH, Younger JB.* Serum immunoglobulin levels during normal pregnancy. Am J Obstet Gynecol 1971;109:971-6.
17. *Holm G, Petterson D, Mellsed H, Hedfors E, Bloth B.* Lymphocyte subpopulations in peripheral blood of healthy persons. Characterization by surface markers and lack of selection during purification. Clin Exp Immunol 1975;20:443-57.
18. *Rukavina D.* A modified test of active T lymphocytes and its clinical significance. Period Biol 1983;85:suppl3:243-6.
19. *Smith RA, Kerman R, Ezdingly E, Stefani S.* A modified assay for the detection of human adult active rosette forming lymphocytes. J Immunol Methods 1975;8;175-84.
20. *Hartzman RJ, Segall M , Bach ML, Bach FH.* Histocompatibility matching. VI. Miniaturization of the mixed leukocytes culture test: a preliminary report. Transplantation 1971;11:268-73.
21. *Morović M, Rukavina D, Sepčić J, Eberhardt P, Ledić D, Herman H.* Comparability of multiple sclerosis and chronic relapsing experimental allergic encephalomyelitis. II. Immunologic observations. Period Biol 1986;88:supp1/A:335-6.
22. *Birk K, Ford C, Smeltzer S, Ryan D, Miller R, Rudick RA.* The clinical course of multiple sclerosis during pregnancy and the puerperium. Arch Neurol 1990;47:738-42.
23. *Iwatani Y, Amino N, Tachi J, Kimura M, Ura I, Mori M et al.* Changes of lymphocyte subsets in normal pregnant and postpartum women: postpartum increase of NK/K (Leu 7) cells. Am J Reprod Immunol Microb 1988;18:52-5.
24. *Sepčić J, Rukavina D, Ledić D, Morović M.* Trudnoća i multipla skleroza: Prikaz slučaja i analiza imunoloških reaktivnosti. Liječ Vjesn 1982;104(5):178-80.
25. *Fugisaki S, Mori N, Sasaki T, et al.* Cell mediated immunity in human pregnancy. Microbiol Immunol 1979;23:899-907.
26. *Hogart PJ.* Immunological aspects of mammalian reproduction. New York, Praeger Publisher 1982;pp83-449.
27. *Birk K, Rudick R.* Pregnancy and multiple sclerosis. Arch Neurol 1986;43:719-26.
28. *Bisset L R, Fiddes TM, Gillett WR, Willson PD, Griffin JFT.* Altered humoral immunoregulation during human pregnancy. Amer J Reproduc Immunol 1990;23:4-9.
29. *Abramsky O, Lubetzki-Korn I, Evron S, Brenner T.* Suppressive effect of pregnancy on MS and EAE. Prog Clin Biol Res 1984;146:399-406.
30. *Keith AB.* Effect of pregnancy on experimental allergic encephalomyelitis in guinea pigs and rats. J Neurol Sci 1978;38:317-26.
31. *Abramski O, Brenner T, Mizrachi R.* Alpha fetoprotein suppress experimental allergic encephalomyelitis. J Neuroimmunol 1982;2:1-7.
32. *Arnason BG, Richman DP.* Effect of oral contraceptives on experimental demyelinating disease. Arch Neurol 1969;21:103-8.

VESNA BARAC-LATAS
MIRO MOROVIĆ
JURAJ SEPČIĆ
NIKOLA MATEJČIĆ
DANIEL RUKAVINA

IMUNOLOŠKA REAKTIVNOST U ŽENA S MULTIPLOM SKLEROZOM TIJEKOM TRUDNOĆE I POSLIJEPORODAJNOG RAZDOBLJA

IZVORNI ZNANSTVENI ČLANAK

Ključne riječi:
trudnoća, puerperij, multipla skleroza, imunologija

Prihvaćeno: 1992-11-25

SAŽETAK *Proučavan je imunološki odgovor u deset žena s multiplom sklerozom (MS) tijekom trudnoće i puerperija. Metodom formiranja rozete utvrđene su subpopulacije limfocita u perifernoj krvi. Koncentracija imunoglobulina u serumima je određivana radioimunodifuzijskom pretragom, a funkcionalna reaktivnost limfocita na poliklonske mitogene metodom po Hartzmanu. Ukupni i aktivni T-limfociti pokazali su značajno niže razine tijekom drugog i trećeg tromjesečja trudnoće. Aktivni T-limfociti bili su na najnižoj razini u poslijeporođajnom razdoblju. Nije bilo značajnih promjena u postotku B-limfocita u trudnica oboljelih od MS nasuprot kontrolnoj skupini. Postotak ukupnih i aktivnih T-limfocita se značajno smanjio u trudnica oboljelih od MS tijekom ponovnog javljanja bolesti. Koncentracija imunoglobulina je bila značajno snižena u trudnica oboljelih od MS nasuprot zdravim ženama koje nisu bile trudne. Koncentracija IgG značajno se smanjila tijekom trećeg tromjesječja trudnoće u trudnica oboljelih od MS; bila je ekstremno niska. Proliferacijski odgovor na mitogene značajno se smanjio u trudnica oboljelih od MS tijekom poslijeporođajnog razdoblja u odnosu prema razdoblju trudnoće. Proliferacijski odgovor na PHA i ConA utvrđen u trudnica oboljelih od MS tijekom ponovnog javljanja bolesti bio je značajno snižen. Očito je da je gestacija povezana s depresijom prije selektivnih negoli općih aspekata staničnog imuniteta. Humoralni imunitet nastavlja normalno funkcionirati tijekom trudnoće, iako su mišljenja o koncentracijama imunoglobulin u krvi trudnica još uvijek proturječna.*