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The Influence of Pregnancy on Development and Course of Chronic Relapsing Experimental Autoimmune Encephalomyelitis in Rats: Implications for Multiple Sclerosis

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A B S T R A C T

Multiple sclerosis is a chronic, autoimmune disease of the central nervous system, which mainly affects young women during a reproductive period of life. Since, its symptoms might be significantly affected by pregnancy, in this study we investigated the development and kinetics of disease in the model of chronic relapsing experimental autoimmune encephalomyelitis (CR-EAE), induced in genetically susceptible Dark Agouti (DA) strain of rats. They were sensitized with bovine brain white matter homogenate (BBH) in complete Freund's adjuvant during the first, second or third week of pregnancy, and the disease scores were compared between treatment groups, and identically treated nongravid females. Additionally, the susceptibility to the induction of EAE was tested in offspring of mothers that during the pregnancy were sensitized with BBH. The data have shown that pregnancy does not block the induction of EAE, but that it significantly changes the course of diseases, depending on time of immunization. In rats sensitized during the first week of gestation the onset of the clinical signs was delayed, but after the delivery the intensity of disease significantly increased. Similar aggravation, with appearance of monophasic form of disease was observed in the group of rats sensitized during the third week of gestation. On the contrary, in rats sensitized during the second week of gestation the beneficial effects were observed, with later onset of attacks, and lower disease score. Furthermore, offspring of these rats after immunization with BBH developed a monophasic form of EAE of lower intensity, suggesting that some protective factors might be transferred across the placenta.

***Key words**: chronic relapsing experimental autoimmune encephalomyelitis, multiple sclerosis, pregnancy, offspring*

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with onset typically in the second to third decade. It is twice as prevalent in females as in males and mainly affects young women during a reproductive period of life[1,2]. Previously, they were often discouraged from having children, owing to the potential exacerbation of MS, but current opinion is that women with MS and healthy women have similar incidences of pregnancy or delivery-related complications[3–5]. Even more, disease activity is almost invariably halted during pregnancy, although after delivery the relapse rate frequently increases[6,7], particularly in the post partum three months[8,9]. Amelioration or a worsening of the disease was mostly related with high, or low-estrogen states, respectively[10].

Some of these changes may be explained by a favorable immunological shift from a Th1 to Th2 response during pregnancy, since estrogens inhibit the production of Th1 proinflammatory cytokines, such as IL-12, TNF-α and IFN-γ, whereas they stimulate the production of Th2 anti-inflammatory cytokines, such as IL-10, IL-4, and TGF-β[9,11,12], but the underlying mechanisms and interactions between the multiple sclerosis and pregnancy are still investigating.

Since, important basic information about the pathogenesis of MS and neuro-immuno-endocrine interaction

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during pregnancy came from the animal models of multiple sclerosis, in this study we attempted to investigate a potential role of prenatal and perinatal pregnancy-related events on the development of experimental autoimmune encephalomyelitis (EAE) in susceptible *Dark Agouti* (DA) strain of rats[13,14], in which chronic relapsing (CR) form of EAE might be induced by immunization of rats with bovine brain white matter homogenate (BBH) in complete Freund's adjuvant (CFA)[15,16]. To elucidate the effects of stage of pregnancy on the development of CR-EAE in mothers, active immunization of DA rats was done in the first, second and third week of pregnancy. Additionally, in some groups the reactivity of offspring to the inducement of EAE was analyzed.

Materials and Methods

Rats

DA rats, aged 6–12 weeks, from our own colony were used. They were fed on a standard commercial food and water *ad libitum*. Female rats were mated with syngeneic males. The presence of microscopically estimated sperm in vaginal smears was considered as the first day of pregnancy. Virgin females of the same age were used as the controls.

Plan of experiments

Immunization was performed in nonpregnant (control group) and pregnant DA rats by subcutaneous injection of 0.1 ml of the bovine brain white matter homogenate (BBH) emulsion in the complete Freund's adjuvant (CFA), which according to our previous data was efficient in the induction of CR-EAE, as previously described[15,16].

In pregnant rats immunization was performed on the 1st, 2nd and 3rd week of pregnancy, i.e. on the 5th, 10th or on the 15th day after matting. Each group consisted of nine females. During the gestation and until the 35–37th day after the delivery, they were inspected daily for the signs of neurological deficits. The data were compared with the findings in age-matched, nongravid female DA rats, which were immunized on the same way.

Further, to assess the effects of immunization of mothers on EAE in offspring, pups of mothers immunized on the second week of pregnancy, were collected and given for nursing to foster mothers. At the age of 6 weeks they were immunized by BBH on the same way as the control, non-gravid females and inspected daily for the sings of EAE.

Assessment of disease

The severity of disease was clinically assessed according to the following criteria: 0 – no symptoms; 1 – flaccid paralysis of tail; 2 – hind legs paresis; 3 – hind legs paralysis with incontinence and 4 – death of the animal. Histological examinations were made by hematoxylin-eosin staining of formalin-fixed sections of brain and spinal cord.

Statistical analysis

Significance of differences between the groups and the controls were determined by Mann-Whitney U test. P values less than 0.05 were considered statistically significant.

Results

The influence of pregnancy on CR-EAE

Control, nonpregnant female DA rats sensitized with the BBH in CFA developed a chronic relapsing form of EAE (control groups on Figures 1 – 4). First signs of disease appeared after a latent period of 8 days and first attack lasted about 5 days. The second, spontaneous relapse appeared on the 18th day and lasted until the 24th day, when the second remission occurred. Two out of 9 rats (22.2%) died during the first acute attack and 3 out of 7 (42.8%) during the relapse. Disease was followed by histopathological changes typical for EAE, such as extensive perivascular infiltrates of mononuclear cells within spinal cord and brain stem (not shown).

All pregnant rats also developed EAE, but significant, time-dependent changes were found in the dynamics of EAE (Figures 1–3). Thus, in females sensitized with encephalitogen during the first week of gestation the first signs of diseases were postponed for 1 or 2 days (Figure 1) and during the first attack only 1 out of 9 rats (11%) died. During this period the intensity of disease was slightly decreased, but few days after the delivery the symptoms of disease aggravated and from 22nd to 30th day a new strong attack developed, with a high mortality (4 out of 8 rats died; 50%). Moreover, the second relapse was significantly higher in intensity in comparison with the control group of rats (Figure 1; $p<0.01$).

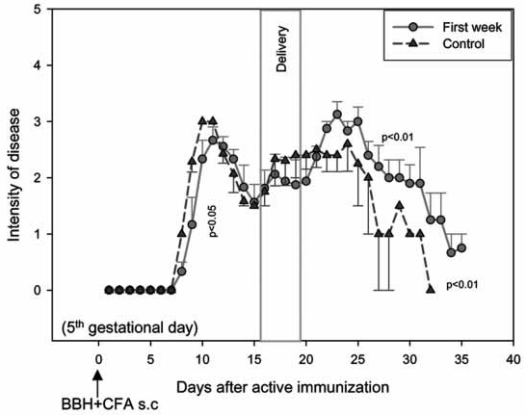

Fig. 1. The clinical course of disease in DA rats sensitized during the first week of gestation, i.e. on the 5th gestational day, by bovine brain homogenate (BBH) in complete Freund's adjuvant in comparison with identically treated non-pregnant DA rats of the similar age. The severity of disease was clinically assessed according to the following criteria: 0 – no symptoms; 1 – flaccid paralysis of tail; 2 – hind legs paresis; 3 – hind legs paralysis with incontinence and 4 – death of the animal. Each group consisted of 9 rats. Data are mean +/– SE.

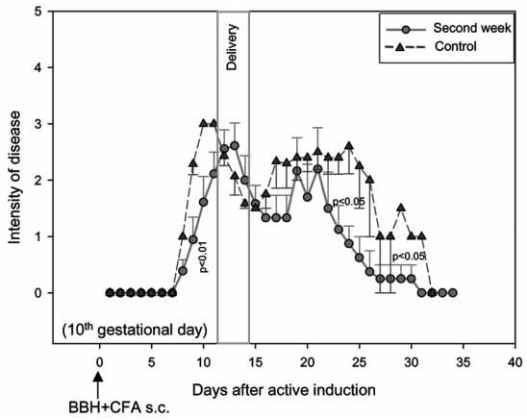

Fig. 2. The clinical course of disease in DA rats sensitized during the second week of gestation (i.e. on the 10th gestational day) by bovine brain homogenate (BBH) in complete Freund adjuvant. Each group consisted of 9 rats. Data are mean +/– SE.

Animals sensitized during the second week of gestation developed the mildest form of disease, characterized by a significant delay in onsets of first and second attack, by a lower intensity of disease and by an earlier termination in comparison with the control (Figure 2). In this group, however, the nonspecific adverse effect of hind legs paralysis on the act of birth was noticed. This led to the death of 4 out of 9 females (44.4%). The rest of 5 animals delivered, but were unable to take care of their offspring, owing to the appearance of clinical symptoms of EAE after the delivery.

In pregnant rats, immunized during the third week of gestation, the first clinical signs of EAE appeared as usual on the 9th day after active induction (Figure 3). Owing to this the delivery was undisturbed and occurred before the onset of the disease. These females, however, in postpartum period developed the monophasic and not the relapsing form of disease. Its clinical score was of high intensity and symptoms lasted for more than 2 weeks. The maximum of the disease was on the 12th day

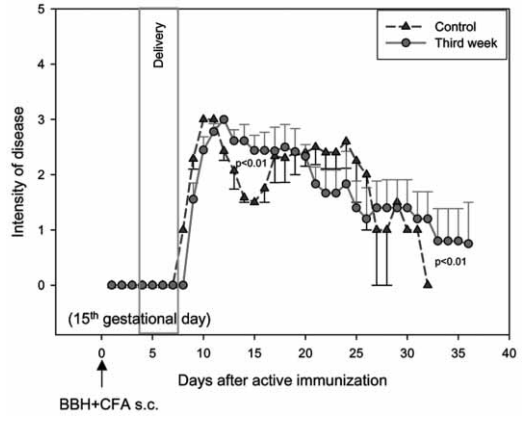

Fig. 3. The clinical course of disease in DA rats sensitized during the third week of gestation (i.e. on the 15th gestational day) by bovine brain homogenate (BBH) in complete Freund adjuvant. Each group consisted of 9 rats. Data are mean +/– SE.

after active induction of disease. Animals completely recovered on the 34th day after active induction, i.e. 2–3 day later in comparison with the control group (Figure 3). Mortality in this group was 55% (5 out of 9 animals died).

EAE in offspring of mothers sensitized during pregnancy

Offspring from mothers immunized during the second week of gestation, were collected and given for nursing to foreign mothers. After sex matching, the group consisted of 10 weaning females rats that survived the stress of delivery and separation from the mothers. At the age of 6 weeks they were immunized with BBH + CFA and the data were compared with standard control (Figures 1–4), consisted of virgin female rats obtained from non-sensitized mothers, which were similarly treated with encephalitogen. As shown in Figure 4 offspring of immunized mothers did not develop the chronic relapsing form of EAE, but the monophasic type of disease, which lasted 2 weeks. The intensity of the disease was lower than in the control, but 44% of animals died. All survived animals recovered completely. On the 32nd day after the first sensitization they were re-sensitized with BBH, but none of them developed the disease (the data are not shown).

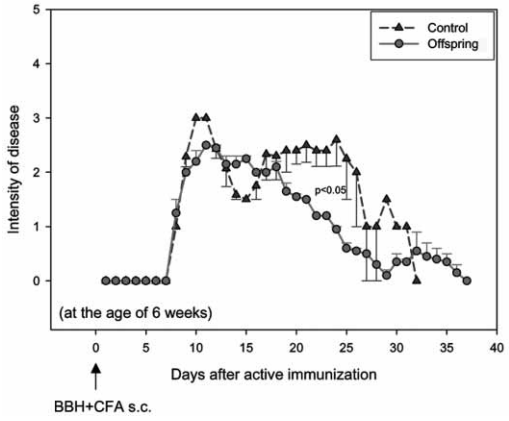

Fig. 4. The clinical course of disease of offspring of DA mothers sensitized during the second week of gestation. Sensitization was made by bovine brain homogenate (BBH) in complete Freund adjuvant at the age of 6 weeks. Young were nursed by milk of foreign, previously non-immunized mothers. Each group consisted of 9–10 female rats. Data are mean +/– SE.

Discussion

The data point to significant interactions between the pregnancy-related events and pathogenesis of EAE, confirming the widespread literature data about the marked influence of pregnancy on the development and clinical stability of multiple sclerosis in humans[1–12,17–19]. The interactions were clearly dependent on the phase of pregnancy, showing that they were influenced by complex neurocrine-endocrine factors that differently affected the afferent, central and efferent arm of immune reaction, activated after the inoculation of encephalitogen. Thus,

in agreement with findings in humans[2,19] and pioneer report of Sepčić et al.[19] in this field, our data underline that pregnancy might have a suppressive and exacerbating effect on acute demyelinating disease. Sensiti- zation of pregnant rats during the first week postponed the onset of EAE (Figure 1), while that made during the second week of gestation resulted in a typical amelioration of disease and it's aggravation after the delivery (Figure 2). Moreover, in agreement with others[20,21] we found that offspring of these mothers developed a monophasic instead of chronic relapsing form of EAE, as well as that survivors were resistant to the re-induction of EAE (Figure 4), suggesting that protection was partially transferred from mother to their progeny. The later data are consistent, but slightly different from results obtained by Dimitrijević et al.[22], who investigated the effects of pregnancy on EAE in the same strain of rats and found the most pronounced suppression of EAE in offspring of mothers, which were immunized before gestation and during lactation and only delayed onset of the disease in DA progeny after immunization of mothers during pregnancy. It should be, however, emphasized that immunization protocol and clinical assessment of disease in our study were different, as well as that we tested only the reactivity of offspring from mothers, immunized during the 2nd week of gestation, which exhibited the most significant suppressive effects of pregnancy on EAE. Moreover, since in the report of Dimitrijevic et al.[22] it was clearly shown that protective effects in progeny might be dependent on lactation and not on passive transfer of anti-myelin basic protein antibodies across the placenta, it is important to emphasize that in our experiments the offspring were nursed by foreign mothers, showing that in their protection participated the additional factors.

Our data need further investigations, but they underline the time dependent effect of pregnancy on clinical course of CR-EAE in DA rats and their offspring. They are in high agreement with data obtained in remitting-relapsing model of murine EAE, and other experimental models[23,24], but unfortunately we can only speculate about the underlying mechanisms, which involve numerous local and systemic pregnancy-induced influences on the development of CR-EAE. Currently, it is generally held that the priming and expansion phase of EAE occurs within the systemic immune compartment, as well as that the processes of restimulation of myelin-reactive T cells occurs in CNS, where encephalitogenic T cells reencounter their cognate Ag, in the context of major histocompatibility complex class II molecules leading ultimately to local inflammation and demyelination[25,26]. Process includes specific interaction between infiltrating T cells and the resident CNS glia cells and numerous regulatory mechanisms that control the inflammation and induce rapid clearance of lymphoid cells early after disease[27]. Many of them are under the control of pregnancy-related factors[1–12, 26]. Thus, supporting the role sex steroids in these events it has been shown that high levels of estrogens and progesterone during EAE might reduce the presentation ability of dendritic cells[28] and induce a favorable immunological shift from a Th1 to Th2 response[11,12,29]. Furthermore, in animal models it was found that estrogen treatment might in spleen and CNS induce the appearance of regulatory T cells with suppressive phenotype[30,31] and upregulated FoxP3 gene expression[32]. Moreover, similar increase in the proportion regulatory T cells was found in the pregnant women with MS[33,34].

Besides, as reported by numerous recent reviews, short-term beneficial effect of pregnancy on the course of MS and EAE might be linked with the activity not only on sex-steroids, but also with other hormones and pregnancy-associated substances that participate in creation of a specific immunosuppressive or immunoregulatory environment[1–5,26,35]. It is likely that among them are also the pregnancy-induced changes in the liver, such as increased production of acute phase proteins, as well as the generation of cytotoxic NKT, which in EAE might have a protective role[36,37]. Emphasizing the participation of these mediators in gestation, on the 16th day of syngeneic pregnancy we found a high accumulation of NKT cells in the maternal liver[38] and a marked upregulation of metallothioneins (MTs) I+II in the liver and at the feto-placental unit. Similarly, during CR-EAE in DA rats we found enhanced expression of MT I+II both in the liver and in the CNS (manuscript in preparation), but although these data suggest that these stress proteins might also influence the outcome of autoimmune disease in mothers and their progeny, this speculation needs to be clarified by further experiments.

In conclusion, in the model of CR-EAE in DA rats we showed that pregnancy-associated events might significantly change the dynamics of disease in mothers, as well as in their offspring, resulting (depending on the time of sensitization) in amelioration of the clinical symptoms (during pregnancy) and in worsening of disease (in the postpartum period). Since CR-EAE is very similar to multiple sclerosis in humans the data might be of importance for the women of reproductive age with MS.

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UTJECAJ TRUDNOĆE NA RAZVOJ I TIJEK KRONIČNOG RELAPSIRAJUĆEG EKSPERIMENTALNOG AUTOIMUNOG ENCEFALOMIJELITISA U ŠTAKORA: IMPLIKACIJE ZA MULTIPLU SKLEROZU

S A Ž E T A K

Multipla skleroza (MS) je kronična autoimuna, demijelinizirajuća bolest središnjeg živčanog sustava, koja najviše pogađa mlađe žene u reproduktivnoj životnoj fazi. Budući da su tijekom trudnoće opisane značajne promjene u simptomatologiji i tijeku MS, u ovom radu smo nastojali primjenom animalnog modela kronično relapsirajućeg autoimunog encefalitisa (KR-EAE) istražiti kako pojedine faze trudnoće utječu na razvoj bolesti. Bolest je inducirana u genetski osjetljivih DA štakora imunizacijom s homogenatom bijele tvari goveđeg mozga (BBH) u kompletnom Freundovom adjuvansu (CFA), koji se injicirao tijekom prvog, drugog ili trećeg tjedna trudnoće. Procjenjivala se klinička slika bolesti tijekom 37 dana nakon imunizacije, kao i mogućnost indukcije EAE u potomaka majki, koje su tijekom trudnoće bile imunizirane na encefalitogen. Rezultati su pokazali da trudnoća ne zaustavlja razvoj bolesti, ali da ima značajan modulacijski učinak na klinički tijek KR-EAE, koji ovisi o vremenu izvršene imunizacije. U ženki senzibiliziranih tijekom prvog tjedna gestacije pojava prvih kliničkih znakova bila je odgođena, ali su nakon okota intenzitet i trajanje bolesti bili značajno veći. Slično pogoršanje kliničke slike, sa pojavom monofaznog oblika bolesti produženog trajanja utvrđeno je i u ženki senzibiliziranih tijekom trećeg tjedna gestacije. Nasuprot tome, u gravidnih ženki senzibiliziranih tijekom drugog tjedna gestacije uočena je najveća odgoda prvih znakova i najkraći tijek bolesti. Osim toga, mladi ovih majki (koje su dojile zdrave ženke), su nakon imunizacije s encefalitogenom u dobi od 6 tjedana, razvili monofazni, a ne kronično relapsirajući eksperimentalni autoimuni encefalomijelitis, sugerirajući da se neki od protektivnih čimbenika prenose transplacentarnim putem.