Short Communication

IN VIVO EFFECTS OF MATERNAL IMMUNOSUPRESSION DURING PREGNANCY ON THE IMMUNE FUNCTION OF NEWBORN INFANTS*

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When used in pregnancy, immunosuppressants can cross the placental barrier and enter foetal circulation, possibly affecting the immune system of the foetus. This study evaluated the immune function in eight children born by mothers with connective tissue diseases who received immunosuppressants (cyclosporine A or dexamethasone) during pregnancy and in six babies from mothers with similar diseases, but who did not receive any treatment. Judging by the cytokine production of interleukin-2 and interferon-γ in peripheral blood mononuclear cells stimulated by phorbol-myristate-acetate (PMA) and ionomycin, immunosuppressive drugs given for rheumatic disorders during pregnancy do not induce significant immunosuppression in babies.

KEY WORDS: *cyclosporine A, dexamethasone, immune function, immunosuppressants, newborns, vaccination*

Ideally, no drug should be used in pregnancy, and immunosuppressive agents in particular. However, transplantation and the treatment of connective tissue diseases often require the use of these drugs both to protect the mother and to ensure successful pregnancy.

The effects of glucocorticoids on the foetus differ from drug to drug; prednisone is inactivated before it can reach foetal tissue while synthetic fluorinated steroids such as betamethasone and dexamethasone are not. Cyclosporine A crosses the placental barrier and can be measured in foetal blood. Literature reports impairments of immunologic development in up to one year old infants (1).

The aim of this open study was to evaluate the immune function of newborns whose mothers had been receiving immunosuppressants for autoimmune disorders during pregnancy.

SUBJECTS AND METHODS

Subjects

In our high-risk pregnancy clinic, all children born to mothers with autoimmune diseases are usually followed until they complete the first year of life. This study included nine babies (four boys and five girls) born to mothers with autoimmune diseases who had been receiving immunosuppressants during pregnancy. These women were affected by systemic lupus erythematosus (n=2), psoriatic arthritis (n=1), and subacute cutaneous lupus (n=1), while two women were healthy. The latter had been treated with fluorinated corticosteroids during pregnancy because of a complete foetal heart block associated with anti-SSA/Ro autoantibodies. One woman gave birth to twins and the other to triplets. The children

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Table 1 *Maternal diagnosis and the therapy of the mothers receiving immunosuppressants during pregnancy.*

Subject no.	Dx	Tx	Daily dose / mg	Duration / weeks	Cumulative dose / mg
1	PsA	Cyclosporine A	2 x 100	37	51800
2	SLE	Cyclosporine A	150	38	39000
3	Healthy	Dexamethasone	4	9	252
4 (twin)	SCL	Dexamethasone	4 → 0.5*	10	173
5 (twin)		Dexamethasone	4 → 0.5*	10	173
6 (triplet)	Healthy	Dexamethasone	4	16	448
7 (triplet)		Dexamethasone	4	16	448
8 (triplet)		Dexamethasone	4	16	448

Dx = maternal diagnosis; Tx = maternal treatment; PsA = Psoriatic arthritis; SLE = systemic lupus erythematosus; SCL = subacute cutaneous lupus.
** 4 mg/day for 4 weeks, than 2 mg/day for 2 weeks, than 1 mg/day for 2 weeks, than 0,5 mg/day for 2 weeks*

were followed in the Paediatric Department of Milan University in 2002-2003. The control group consisted of 14 children (five boys and nine girls) born to mothers with autoimmune disorders (mainly systemic lupus erythematosus and Sjögren syndrome) who had not been receiving immunosuppressants during pregnancy. Table 1 shows the diagnosis and the immunosuppressive therapy for mothers who received it during pregnancy. Mothers of two children (nos. 2 and 3) were also taking prednisone, while one (of child no. 3) was also taking hydroxychloroquine.

Mothers of infants in the control group were not taking medications for their underlying disorder during pregnancy, but some received hydroxychloroquine (four mothers), baby-aspirin or a low-dose (≤10 mg day^{-1}) prednisone (mostly inactivated before reaching the foetus).

Methods

Eight exposed infants and six controls were tested for interleukin-2 (IL-2) and interferon-gamma (IFN-γ) production by peripheral blood mononuclear cells using the quantitative sandwich ELISA method (R&D Systems, Minneapolis, MN, USA). Cytokine concentrations were evaluated in supernatants from unstimulated or 48-hour stimulated cell cultures (phorbol-myristate-acetate, PMA, 5 ng mL^{-1}, ionomycin, 500 ng mL^{-1} and PMA + ionomycin). Informed parental consent and approval by the hospital ethical committee were obtained for the study. The results of the exposed and control subjects were compared using the chi-square test with correction for multiple comparisons where appropriate.

RESULTS

No significant difference in any of the parameters evaluated was found between the infants of mothers who were receiving immunosuppressive therapy during pregnancy and those who were not (2). Complete blood count was normal in all subjects, except for two infants with anaemia (one exposed and one control) and one with a mild thrombocytopenia (exposed). According to the chi-square test, lymphocyte subpopulations, immunoglobulin serum levels, and IgG subclasses did not significantly differ between the exposed and control group. All infants who received at least one dose of hepatitis B vaccine (recombinant hep B) reached a protective antibody titre (>10 mIU mL^{-1}) (2). Figures 1 and 2 show our preliminary laboratory findings for cytokine (IL-2 and IFN-γ) production by peripheral blood mononuclear cells in the exposed subjects and controls. Even though the number of subjects is small, these data seem to suggest that cytokine production might be impaired in infants exposed to cyclosporine *in utero*.

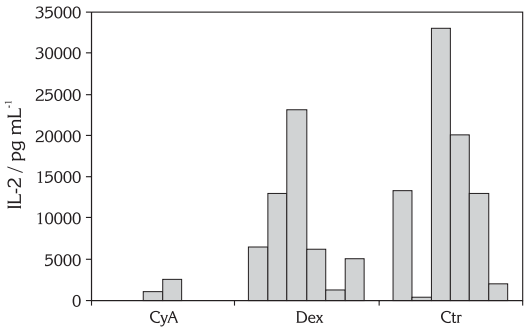

Figure 1 *Individual IL-2 production (pg mL^{-1}) by peripheral blood mononuclear cells stimulated with phorbol-myristate-acetate + ionomycin. Each bar represents one subject: CyA - subjects exposed to cyclosporine A in utero; Dex - subjects exposed to dexamethasone in utero; Ctr – control subjects.*

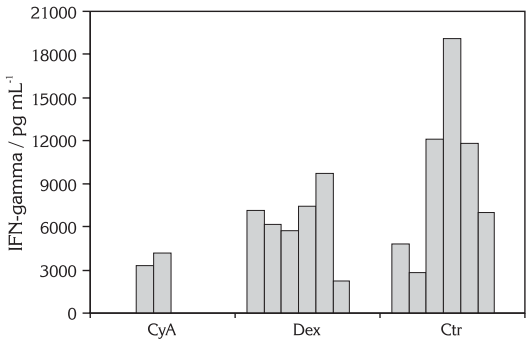

Figure 2 *Individual IFN-γ production (pg mL^{-1}) by peripheral blood mononuclear cells stimulated with phorbol-myristate-acetate + ionomycin. Each bar represents one subject: CyA - subjects exposed to cyclosporine A in utero; Dex - subjects exposed to dexamethasone in utero; Ctr – control subjects.*

DISCUSSION

Immunosuppressive drugs given to pregnant women usually cross the placental barrier and pass to the foetus. Immunosuppressed pregnant women run an increased risk of both foetal and maternal complications (premature birth, intrauterine growth retardation, congenital malformations and pre-eclampsia, respectively). The effects of immunosuppressive and anti-rheumatic agents on pregnancy have been reviewed extensively (3-10).

Until recently, the effects of immunosuppression on foetal and neonatal immune system have not been studied, and most of the published data refer to transplant recipients (11-13). We therefore conducted a pilot study in order to evaluate these effects in children born from patients with autoimmune disorders, and found no relevant effect on their immune function, as evaluated by immunoglobulin concentrations, lymphocyte subpopulations, and response to hepatitis B vaccination.

Our study included children whose mothers had been treated with corticosteroids as well as children who were exposed to active dexamethasone in order to treat their congenital heart block. Cyclosporine A (CyA), an immunosuppressant used mainly in transplantation, can readily cross the placental barrier, and foetal plasma levels can reach more than half the maternal serum levels (14). Several authors studied the effects of CyA (15-23) and of a related compound tacrolimus on pregnancy (24, 25). Cyclosporine A is also excreted in breast milk and can be found in neonatal blood after nursing (26, 27). *Padgett and Seelig* (28) studied the lactational transfer of CyA in an animal model, and found significant alterations in T-cell maturation and the inhibition of lymphoproliferative response to antigen activation, indicating a potential for an increased risk of opportunistic infections. Pilarski *et al.* (29) analysed peripheral blood lymphocytes collected from infants exposed to cyclosporine A and azathioprine *in utero* and found alterations in activated and memory T-cell count. *Di Paolo et al.* (1) also studied lymphocyte subpopulations and immunoglobulin levels in infants exposed to cyclosporine *in utero*. Total B-cell and T-cell counts (as well as CD4+ and CD8+ cell counts) were low at birth, but normalised thereafter. T-cell activation markers such as the alpha chain of interleukin-2 receptor (CD25) and HLA-DR expression were low throughout the study period (up to one year of age). IgG serum levels were normal at birth, but IgA and IgM concentrations were low. In the second month of age, IgG1 and IgG3 subclass levels were low, and remained low up to the sixth month of age. These infants did not develop opportunistic or chronic infections and had a normal growth during their first year of life. However, the authors suggest that conventional vaccination should be postponed in these infants. In our study, the immune parameters of the exposed infants, some of whom to Cyclosporine A, kept within the normal range, and the antibody response to hepatitis B vaccination was normal in all cases, which suggests that the immunologic response to vaccines is not impaired (2). A normal IgG antibody response to vaccination has been demonstrated earlier (30), which is in agreement with our results. Moreover, a recent case report describes normal immune development, including normal serum immunoglobulin levels and normal response to tetanus and hepatitis B vaccinations in a child exposed to Cyclosporine A *in utero* (31).

Literature also describes immunosuppressed newborns exposed to azathioprine *in utero*. This drug is known to cross the placental barrier (32-35). Notably, the foetus is protected from its effects during organogenesis, since it lacks the liver enzyme that converts azathioprine to its active metabolites. As Cyclophosphamide is contraindicated during pregnancy, there are no studies available on its effects. While methotrexate is also contraindicated during pregnancy, there have been case reports of successful pregnancies in women who inadvertently got pregnant while on this drug.

Exposure to immunosuppressants *in utero* raises the issue of possible increased susceptibility to

autoimmune diseases later in life (36-38). In fact, it might impair the ability of the foetal immune system to delete autoreactive T-cells, and immunosuppressants such as CyA have been shown to be able to break self-tolerance by interfering with the production of CD4+ and CD8+ cells in the foetal thymus. In addition, CyA can inhibit the activation of potentially self-reactive T-cells, thereby down-regulating these "autoimmune" clones. On the other hand, discontinued exposure could allow autoimmunity to develop and progress. Indeed, lethally irradiated rodents exposed to CyA during immune reconstitution (post-syngeneic bone marrow transplant) developed a T-cell-mediated autoimmune disease (39). Another report refers to a case of ulcerative colitis and systemic lupus erythematosus in a girl exposed to azathioprine *in utero* (40). The effect of altered thymocyte differentiation during negative selection of autoreactive T-cells that was seen in rodents appeared to be related to the down-regulation of thymic MHC class II molecules (39,41). Until now, none of the children in our study have developed new autoantibodies.

The developing immune system has been shown to be affected by maternal cortisol secretion, resulting in an increased susceptibility to asthma and atopy in genetically predisposed individuals (42). This has been thought to be secondary to a Th1/Th2 differentiation imbalance, and steroid treatment during pregnancy, particularly when the fluorinated steroids are used, could theoretically drive the balance toward a Th2-like response (43, 44). We will therefore continue to follow the children described in this study for possible development of autoimmune and/or atopic disorders during their childhood and adolescence.

CONCLUSION

Immunosuppressive therapy of rheumatic disorders during pregnancy does not seem to induce significant immunosuppression in babies. However, this issue requires further investigation. Data from long-term follow-up of infants exposed to immunosuppressants *in utero* are still too limited to exclude possible development of autoimmune diseases later in life.

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Sažetak

IN VIVO UTJECAJ IMUNOSUPRESIJE U MAJKI ZA VRIJEME TRUDNOĆE NA IMUNOSNI SUSTAV NOVOROĐENČADI

Imunosupresivni lijekovi davani za vrijeme trudnoće mogu proći placentalnu barijeru i ući u cirkulaciju fetusa, s mogućim utjecajem na njegov imunosni sustav. U radu je praćena imunosna funkcija kod osmero djece rođene od majki s bolestima vezivnog tkiva, koje su tretirane za vrijeme trudnoće imunosupresivnim lijekovima (ciklosporin A ili deksametazon) i kod osmero novorođenčadi rođene od majki sa sličnim bolestima, ali koje nisu bile tretirane. Imunosupresivni lijekovi primijenjeni za vrijeme trudnoće kod majki koje boluju od reumatskih bolesti ne izazivaju značajniju imunosupresiju u novorođenčadi praćenu nastajanjem citokina, interleukina 2 i interferona γ u perifernim mononuklearnim krvnim stanicama pod djelovanjem forbol-miristat-acetata (PMA) i ionomicina.

KLJUČNE RIJEČI: *ciklosporin A, deksametazon, imunosupresivni lijekovi, imunosna funkcija, novorođenče*

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