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1α,25-dihydroxy-vitamin-D3 as new immunotherapy in treatment of recurrent spontaneous abortion

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Summary Recurrent spontaneous abortion (RSA) is serious health problem affecting 2–5% of reproducing couples worldwide. It has long been suspected that nearly 80% of the unexplained RSAs are due to immunologic causes. Although the major tissue confronting the mother's immune system is the placental villous trophoblast, the immunological risk to the developing embryo is not great until the time of implantation. In addition, trophoblast is not sensible to lysis by NK cells, TNF-α or macrophages, but may be killed by lymphokine activated NK cells (LAK) and may undergo apoptosis in response to TNF-α and/or IFN-γ in vitro. The two most commonly used treatments for RSA are intravenous immunoglobulin (IVIg) and alloimmunization with partner's leukocytes (LIT). We promote vitamin D3 as new immunomodulatory agent in treatment of RSA. Different mechanisms have been proposed to account for the immunosuppressive effect of 1α, 25-dihydroxy-vitamin-D3 (VD3). Portion of the VD3 activity involves the downregulation of IL-2, IFN-γ and TNF-α genes transcription. Because immunomodulatory effects of VD3 are very similar to IL-10 effects, acting of VD3 in immunotherapy of RSA syndrome, preeclamptic and eclamptic pregnancy, as well as PIH syndrome, is very reasonable. We propose using of VD3 as immunotherapy or adjuvant therapy in combination with classic immunotherapies of endangered pregnancies.

Mechanisms of recurrent spontaneous abortion

In most mammals, cells of the syncytiotrophoblast are totally MHC I and II negative, but the underlying cytotrophoblasts are weakly MHC I positive [1]. Extravillous cytotrophoblasts express a kind of MHC class I molecules, so-called HLA-G and its expression is limited to the placenta and the epithelial cells of the thymus [2]. The HLA-G positive placental cells play important immunoregulatory role, as they are much less sensitive to NK cell mediated lysis. In addition, HLA-G may confuse, trap or defuse the T cell receptor (TCR) complex. As none of the forms of trophoblast carries class II MHC molecules they cannot stimulate T helper cells directly to begin immunological reactions. Consequently, without any Class I or II MHC molecules, the villous trophoblast cells cannot function as the targets for MHC directed cytotoxic T cells [2,3].

Cytokines are seen to have a complex role in post-implantation pregnancy. In normal pregnancy, trophoblastic cells are resistant to lysis by

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cytotoxic T lymphocytes, NK cells and antibody dependent cytotoxicity [3]. The lymphokines, which can activate NK cells into LAK cells, include TNF-α, IL-2, IFN-, IL-12 and IL-18. Another cytokines have been shown to prevent LAK cell activation and abortion. These include IL-3, GM-CSF, CSF-1, IL-10 and TGF-β produced by CD8+ cells expressing progesterone receptors [3,4]. TGF-β has been shown to be a competitive antagonist of IL-2, where IL-2 induces the activation of NK cells and the secretion of TNF from these cells [4,5].

T helper type 1 (Th1) cells produce abortogenic cytokines such as IL-2 and INF-γ which are seen to cause abortion in mice; T helper type 2 (Th2) cells however produce IL-3, IL-4 and IL-10 which promote antibody formation, put off inflammation and NK cell activation [6—8]. The NK derived INF-γ may activate the macrophages of the feto-maternal interface or other TNF-α secreting cells whereas Th2 cells would suppress this activation [4,5,8].

Some authors represent opinion that the major inhibitor of the abortogenic reaction of the NK cells is seen to be the trophoblast dependent natural suppressor (NS) cell. During pregnancy, there are an increased number of NK cells in the decidua and these cells account for about 40% of the decidua lymphocytes. These cells are uniquely CD56+CD16− and are seen to produce suppressor factors and Th2 cytokines. It is possible that these NK cells are NS cells, which are responsible for maintenance of the fetal allograft and initiating the appropriate immune response [6,7,9].

The CD8+ T cells with the αβ-TCR appear to be the more protective T cell subset. Production of IL-4 and IL-7 by the trophoblast would be expected to deviate T cell differentiation along the Th2 pathway whatever the TCR phenotype and along with the trophoblast cell derived factors, such as GM-CSF, may boost NS cell activity. Independently, IL-10 and IL-4 may inhibit NK cell activation into LAKs [8,10]. In these processes, maybe the most important mechanism is extrathymic lymphocyte maturation pathway. In pregnancy, the site of extrathymic lymphocyte maturation is decidua [5,6,10].

The basis for pregnancy failure is thus centered on the activation of the NK cell into a LAK cell. Some of the data obtained from studies of RSA seem to suggest that abortion occurs due to failure of the activation of NS cells. These cells carry the γδ-TCR rather than the αβ-TCR that requires HLA-A, B and C for efficient recognition and binding [11]. As well as showing a deficiency in CD56+CD16− cells, women experiencing RSA show an increased level of the CD56+CD16+ NK activated cells in the decidua and blood [2,3].

The cytokines TNF-α and IFN-γ play an important role in abortions, as their administration increases the abortion rate and specific antagonists decrease the abortion rate. It has been proposed that macrophage derived TNF-α stimulates NK cells to produce IFN-γ, which further activates the macrophages, as occurs in the early defense response to infectious agents. There are other potential sources of TNF-α and IFN-γ, and systemic Th1 type responses may cause abortions via augmenting levels of such cytokines; IL-2 may also cause abortions by contributing to NK macrophage activation at the feto-maternal interface. TNF-α is thought to be part of the mechanism, which brings about pregnancy loss through its effects on the placenta. In women experiencing RSA, circulating IFN-γ promote mechanisms of MHC class I and II molecules expression. Consequently, MHC expressing cells become apparently target cells. In addition, IFN-γ stimulated macrophages produce TNF-α. This in effect would cause the recognition of the induced MHC expression and thus rejection of the fetal allograft by the maternal immune system would result [4—6]. Maternal recognition of the conceptus as foreign is seen by some as the primary or possibly the only step in preventing its rejection. In animal models, TNF-α and IFN-γ coadministration aborted >80% of the embryos, whether or not NK cells or macrophages had been depleted or estradiol and progesterone was injected to correct potential reduction in ovarian function by cytokines. Some authors have shown that the embryos die from ischemia due to activation of vascular endothelial cell procoagulant, which causes thrombosis and inflammation [6,8,9]. This appears similar to the mechanism whereby TNF-α causes ischemic necrosis of nonantigenic tumors [12].

Current models of immunotherapy in patients with RSA past

Various forms of immunotherapy have been introduced to treat couples suffering from recurrent unexplained abortions. IVIg is seen to suppress anti-phospholipid antibodies and is the therapy used when conventional anti-coagulant or immunosuppressive treatment is ineffective. It has been noted that the IVIg infusion contains anti-idiotypic antibodies, which inhibit the binding of anti-phospholipid antibodies to corresponding antigens and inactivate idiotype bearing B cells. Alteration of T cell subsets, modulation of cell mediated responses, and blockade of the immunoglobulin FcgR on monocytes, as well as reduction of the NK cytotoxicity

have been reported [13]. Down-regulation of CD56+ and CD56+ CD16+ NK cells have been seen in women treated with IVIg infusion. The infusion is effective in enhancing the percentage of live births among women experiencing RSA. Recent data suggest that IVIg therapy is useful in maintaining pregnancies among women with a history of RSA who lose karyotypically normal embryos and who demonstrate elevated levels of circulating NK cells [11,13].

The application of LIT involves immunizing the mother with leukocytes from either paternal or third party origin. The LIT has been implicated in an attempt to produce a maternal immunoglobulin effectors believed necessary for pregnancy maintenance [5,14]. The foundations for LIT is composed of three suppositions; (a) there is a maternal immune response to the conceptus that develops in all pregnancies that must be blocked, (b) blocking factors develop in all successful pregnancies and (c) in the absence of blocking antibodies, rejection of the fetus occurs [5,14].

The rationale for using seminal plasma in treatment of RSA is provided in the concept that the mammalian female responds to antigens present not only on the trophoblast but also in seminal plasma. The antiphospholipid syndrome, in women who suffer from RSA, has been successfully treated using aspirin, heparin, and prednisone or combination of the three [5–7].

Immunomodulatory effects of VD3

Over the past decade, clinical evidence has been accumulating that VD3 and its analogs are effective in the treatment of Th1 immunity mediated disease. Our opinion is that RSA is also Th1 immunity disease. The mechanism of VD3 activity, however, is not yet fully understood since this vitamin is pleiotropic. VD3 is thought to exhibit anti-inflammatory properties, and has been shown to inhibit T cell proliferation and the production of cytokines, such as interleukin IL-2 and interferon IFN-γ, and TNF-α. Some authors have already reported that VD3 downregulate the production of inflammatory cytokines, such as IL-1, IL-6 and IL-8, stimulated with TNF-α and IFN-γ [15,16].

Human naive Th and cytotoxic (Tc) T cells, which only produce IL-2, may differentiate into Th1/Tc1 or Th2/Tc2 like lymphocytes, characterized by their cytokine production profile. VD3 has been reported to inhibit Th1/Tc1 related, but increase Th2/Tc2 associated cytokines in T cells from adults. VD3 also inhibits not only IL-12 generated IFN-γ production, but also suppresses IL-4 and IL-13 expression induced by IL-4 [17,18]. The T cell response to alloantigen is dependent on T cell receptor activation and costimulation via engagement of CD28 and CD40. A short treatment with fusion proteins and antibodies disrupting these co-stimulatory pathways has been shown to prevent indefinitely acute and chronic allograft rejection in rodents and primates, stimulating the search for low molecular weight compounds able to achieve tolerance induction by co-stimulation blockade. The unique capacity of dendritic cells (DC) to activate naive T cells correlates with elevated expression of MHC antigens and costimulatory molecules, rendering them attractive targets for co-stimulation blockade. VD3 inhibits the ability of antigen presenting cells (APCs) to induce T cell activation and downregulate APCs costimulatory molecules expression [15,18]. Treatment of human DC during their differentiation from monocytes in the presence of GM-CSF and IL-4 with VD3 inhibited markedly the expression of CD80, CD86 and CD40, and partially of class II MHC molecules, leading to an immature DC phenotype characterized by high mannose receptor and low CD83 expression. The inhibitory effect of VD3 on DC maturation was comparable to that induced by IL-10, a cytokine which inhibits APC at different levels, including secretion of IL-12 [16,18]. The reduced expression of class II MHC and co-stimulatory molecules decrease the capacity of DC to activate alloreactive T cells, as determined by the decreased proliferation and abrogation of IFN-α secretion in MLR. These results suggest that the ability of VD3 to decrease expression of co-stimulatory molecules on human DC might contribute to its inhibitory effect on APCs dependent T cell activation and its immunosuppressive properties in allograft and trophoblast rejection. In the absence of ConA stimulation, peripheral blood mononuclear cells (PBMC) did not secrete detectable levels of IFN-γ. When ConA was employed in the stimulation of the cells, these cells synthesized detectable levels of IFN-γ [18]. If VD3 was presented in the medium, the vitamin significantly suppressed the ConA stimulated IFN-γ production by PBMC. In the absence of ConA, PBMC secreted small amounts of TNF-α. VD3 significantly downregulate the ConA stimulated TNF-α by PBMC. Pichler et al. [18] used RT-PCR to investigate the effects of VD3 on the transcription of cytokines [18]. Although IL-6 and IL-8 mRNA was detected in the freshly isolated PBMC, in the PBMC cultured for 24 and 48 hrs in the absence of ConA stimulation, VD3 decrease the expression of IFN-γ, IL-8, TNF-α, IL-2 and IL-6 mRNA to below detectable levels [17,18].

Because effects of VD3 are very similar with immunomodulatory effects of IL-10, we have tested

VD3 on several patients with RSA in preparation for the next pregnancy. We treated the patients with VD3 in doses of 5—10 μg/kg of body weight, with or without immunosuppressive/anticoagulant therapy. First results of our investigation are very encouraging. We also believe that VD3 can be usable as local immunomodulatory drug. Actually, we have opinion that VD3 can be used for direct treatment of endometrium in preparation for the pregnancy. Furthermore, VD3 can be used as immunomodulatory agent in preparation for IVF/ET or treatment of preeclamptic and eclamptic patients.

Conclusion

Pregnancy is a complex integration of the body's endocrine, neurological, anatomical and immunological systems as well as having significant influence from the environment. Immunological mechanisms of tolerance or intolerance to the placental unit are still only partially explored. The choice and designing of appropriate immunotherapeutic procedure in treating RSA may, therefore, pose a serious problem for practitioners. For the same reasons, different immunotherapies have been suggested for the immunological mechanisms involved in RSA and evidence from clinical trials has shown their relative success. VD3 is relatively new immunomodulatory agent with pleiotropic nature. Part of the immunomodulatory effects of VD3 is established on stimulation of Th2 immunity, suppression of MHC and co-stimulatory molecules expression, as well as Th2 cytokine promotion. Because RSA is Th1 phenomenon (in most cases), using of VD3 in prevention or treatment of RSA, or preeclamptic and eclamptic pregnancy may be very useful. To that effect, our first experiences are very encouraging.

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