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Abstract

The immunological paradox of nurturing a fetus with paternal antigens poses some perplexing questions. Peter Medawar, an immunologist, asked at a lecture, "How does the pregnant mother contrive to nourish within itself, for many weeks or months, a fetus that is an antigenically foreign body?" Researchers have since then struggled to answer this question. The research on this topic has led to a few general hypotheses that try to explain this phenomenon. The downregulation of T cells toward paternal alloantigens is an accepted hypothesis. Another hypothesis discusses the significance of the decidua and its ability to impair dendritic cells, which are potent antigen presenting cells and critical in initiating an immune response (Ehrlbacher, 2010). Mechanical barrier and cytokine-shift hypotheses also attempt to explain the "riddle of the fetal allograft." Research is ongoing as there is no one clear answer to this query. Some of these hypotheses have flaws in them while others don't explain enough in regard to the safety of a woman and her fetus. There is one hypothesis that appears to hold the greatest significance in understanding the maternofetal relationship and the successful births of millions of children each year: local active suppression in the decidua.

Introduction

Most living organisms have some form of an immune system that protects it from foreign entities. Humans have a complex immune system that protects us on a daily basis. The notion that an antigenic foreign entity can exist within a human for many months and develop is astonishing. However, this occurs daily with millions of pregnant women across the globe. More than fifty years ago, Peter B. Medawar raised the question of how a semi-foreign transplant with paternal antigens can survive the immune system. Seventy years later, a few hypotheses and the resultant research is available for analysis. This paper will explore and evaluate these hypotheses.

Methods

Information discussed in this paper was compiled from various published articles, taken from Touro's database, including Proquest Science, EBSCO, and PubMed, or Google searches. This paper compares and contrasts the various hypotheses explaining the immunological paradox of a pregnancy and evaluating which are the most siignificant.

Discussion

The Immune System

The main purpose of the immune system is to identify non-self from self and defend the body against non-self proteins, viruses, bacteria, fungi, parasites, tumors and other pathogens. Substances that the body doesn't recognize as its own can activate the immune system. These are called antigens. Proteins on the surfaces of bacteria, fungi and viruses are examples of antigens. When these antigens attach to special receptors on the immune cells (immune system cells), a whole series of processes are triggered in the body. Once the body has come into contact with a disease-causing germ for the first time, it usually stores information about the germ and how to fight it. Then, if it comes into contact with the germ again, it recognizes the germ and can initiate a quicker attack. The body's own cells have proteins on their surface as well: self proteins. Sometimes the immune system mistakenly thinks that the body's own cells are foreign cells. It then attacks healthy, harmless cells in the body. This is known as an autoimmune response (IQWIG 2020).

As soon as the body recognizes a "non-self" entity the immune system will set out to remove the pathogen from the body. The immune system consists of many parts that work together to defend the body against invaders. Two separate, but interacting defensive systems that provide an array of defensive weapons are innate and adaptive immunity. An innate response, neutralizes invading pathogens before they can harm the body. For example, in a wound, white blood cells known as macrophages engulf invading microorganisms as well as release cytokines, signaling proteins, to activate other parts of the immune system. Natural killer cells are an example of immune cells that destroy any pathogen in its path with no need for prior exposure to the invader (Mor, 2007). Adaptive immunity is a more specific response toward a pathogen and is acquired. Activated T and B lymphocytes are specialized white blood cells that are involved in dealing with specific antigens. Antigens (Ag), molecules on foreign organisms that contain distinct epitopes or sites that can initiate an immune response, activate B cells and T cells that "remember" the specific antigens thereby reacting quicker and more vigorously toward the pathogen (Sela, 1998).

The Initial Stages of Pregnancy

After an egg is fertilized it forms a blastocyst that is implanted in the uterus. The sphere consists of an inner cell mass, which becomes the fetus after 8 weeks of conception, and an outer layer that forms the trophoblasts (Mor, 2007). Trophoblasts aid in implantation within the uterus. Syncytiotrophoblasts are multinucleated trophoblasts that form finger-like projections reaching into the mother's bloodstream thereby forming the placenta and aiding in nutrient and gas exchange. With this, understanding the conceptual framework of reproductive immunology is redefined. The trophoblast cells are the only part of the differentiating blastocyst that interacts directly with the mother's immune system. The embryo itself—and

the fetus to which the embryo gives rise—has no direct contact with maternal immune cells. As a result, the real puzzle is not why the mother's immune system tolerates the fetus, but why it tolerates the trophoblast cells (Colbern, Main, 1991).

Proposed Hypotheses

Downregulation of T cells

One study attempted to prove the hypothesis of downregulation of T cells. They discovered that macrophages, an important immune cell involved in antigen presentation, can disable killer T-cells. This, in turn, will prevent the T cells from attacking any object that is recognized as non-self (Anonymous, 1999). In order for this to occur, the syncytiotrophoblasts in the placenta produce an enzyme known as indoleamine 2,3-dioxy-genase (IDO). The function of IDO is to inactivate tryptophan, an amino acid required by T cells to destroy a foreign object (Munn, 1999). In 1990, Andrew Mellor, a colleague of Munn, concluded that IDO inhibits a mother's T cell response towards a genetically different fetus. Miscarriages occurred in the absence of IDO. (Munn et. al., 1998).

In another study he and his colleagues conducted experiments to prove their hypothesis. They used two groups of pregnant mice; one group had been bred to genetically identical fathers of the same strain while the second group was bred to fathers from a genetically different strain (Munn, 1998). The experimenters then embedded time release-capsules consisting of either 1-methyl tryptophan, which is an IDO inhibitor, or a control substance underneath the skin of the pregnant mice. Results showed that only the mice carrying genetically different fetuses that had been given the inhibitor rejected their fetuses (Munn, 1998). Interestingly, the embryos developed normally until inflammatory cells migrated to the implantation site and caused hemorrhaging around the embryo. Munn proposed "the mother is rejecting the placenta and eventually the embryo chokes off and dies" (Munn, 1994). From the data collected, they concluded that after implantation, an embryo starts making connections with the mother's blood supply. Sequentially, syncytiotrophoblasts will start producing IDO, destroying tryptophan and suppressing the maternal immune system (Munn, 1998).

To support the above hypothesis, researchers showed that the antigen receptors of maternal T lymphocytes that recognize paternal alloantigens are specifically downregulated during pregnancy, reducing their ability to initiate an immune response against the fetus (Simpson, 1996). During pregnancy, a semi-allogeneic fetus survives despite the presence of maternal T cells specific for paternally inherited histocompatibility antigens. A mouse transgenic for a T cell receptor recognizing the major histocompatibility (MHC) antigen H-2Kb was used to follow the fate of T cells reactive to paternal alloantigens. In contrast to syngeneic and third-party allogeneic pregnancies, mice bearing a Kb-positive embryo had reduced numbers of Kb-reactive T cells and accepted Kb-positive tumor grafts (Tafuri et. al., 1995). T cell responsiveness was restored after delivery. Thus, during pregnancy maternal T cells acquire a transitory state of tolerance specific for paternal alloantigens.

However, other researchers have reservations about this hypothesis. In "Pregnancy Reconceived", Mor argues that if the maternal immune system is suppressed, exposure to any pathogen would be fatal (Mor, 2007). With evidence of Kb-positive tumor grafts growing in mice (because of its commonality with paternal alloantigens and the risk of a pathogen) it would be nearly impossible for a mother and its fetus to survive. Research showed that presence of immune cells at the implantation site is not associated with a response to the 'foreign' fetus but to facilitate and protect the pregnancy. Therefore, the immune system at the implantation site is not suppressed, on the contrary it is active, functional and is carefully controlled (Mor, 2010).

Mechanical Barrier

Another hypothesis explains that the fetal tissue is unrecognizable as "nonself" by the mother's immune cells due to a mechanical barrier (Mor, 2007). Syncytiotrophoblasts around the fetus contribute to the mechanical barrier between the uterus of a pregnant mother and the rest of her body. This trophoblast-immune interaction includes three stages. Stage one is attraction, the trophoblast cells secrete chemoattractants that will signal immune cells to migrate to the implantation site. The area of the uterus in which the trophoblasts invade is referred to as the implantation site. Stage two, recruitment and/or education, the trophoblasts produce cytokines that regulate the differentiation of immune cells. Upon completion of these two steps, the response can take place. In this final stage, the immune cells from stage two respond to different signals (Swain, 2013). After completion of all three stages, the mechanical barrier is formed. This in turn prevents the movement of activated T cells from the periphery to the implantation site and enables antigens that are inside the barrier to be undetected by the mother's immune system.

In conflict with this hypothesis, researchers have found that the barrier between fetal and maternal is limited at best- fetal cells can be found in the maternal circulation and vice versa, indicating that there is only a partial physical separation between mother and fetus. Furthermore, although placental trophoblasts have reduced antigenicity and attenuated expression of MHC genes, an array of

transplantation antigens is clearly expressed (Fernandez et. al., 1999). A study showed that women produce antibodies and exhibit lymphocyte reactivity against fetal human leukocyte antigens (HLA) (Hunt et. al., 2003). This forces us to recognize that the immune system is not ignorant of, but instead recognizes and responds to these antigens.

Cytokine Shift Hypothesis

The immune system can generally be divided into the innate and adaptive immune system. The former is a nonspecific system providing immediate defense against pathogens, while the latter is more targeted, consisting of T and B lymphocytes. Although communication between these lymphocytes exist, B cells and their antibodies mainly contribute to humoral immunity, whereas T cells primarily provide cell mediated immunity (Abrams, Miller, 2011). T helper cells (CD4+) form a subset of T cells and can be further subdivided, depending on their cytokine production, into T helper 1 cells (Th1) and T helper 2 cells (Th2). Th1 cells secrete interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) which are proinflammatory cytokines, whereas the Th2 cells secrete interleukin 4 (IL-4), IL-10, and IL-13, anti-inflammatory cytokines (Sykes et. al, 2012). A mutually exclusive interaction exists between the Th1 interleukin, IFN-γ, and the Th2 interleukin, IL-4. IL-4 is the dominant factor for promoting growth and differentiation from the Th0 to the Th2 subtype, and directly inhibits the development of the Th1 cells (O'Garra and Arai, 2000). IFN-γ indirectly promotes Th1 differentiation by upregulating the IL-12 receptor whilst inhibiting the growth of Th2 cells (O'Garra, 1998).

Pregnancy is a complex immunological state and this hypothesis suggests that a shift towards T helper 2 (Th2) protects the fetus. When a foreign object enters the body of a woman who is not pregnant, Th1 cells will secrete proinflammatory cytokines that will signal for a cel-mediated response to occur. However, according to the cytokine-shift hypothesis, the balance of Th1 and Th2 will go towards the secretion of cytokines by Th2, resulting in a suppressed inflammatory response (Mor, 2007). Additionally, inhibition of the production of TNF-β and IFN-γ is aided with IL-4 secreted by Th2 cells. The production of IFN-γ, TNF-β and IL-2 are believed to be damaging to pregnancy. In an experiment studying pregnant mice, these cytokines were injected into the mice and caused fetal loss (Koch and Platt, 2003).

Many studies have been conducted in an effort to better understand this cytokine shift. In an experiment conducted by Sykes, et. al. (2012), subjects included pregnant women at 28 weeks and term (prelabour and in labour) and nonpregnant women of child bearing age. The experiment consisted of the isolation of Peripheral Blood Mononuclear Cells (PBMCs). In this study, they employed flow cytometry to examine the effect of stimulation by the mitogen PMA/ionomycin on cytokine production at different gestational stages of pregnancy and during labour (see Figure 1). Th1 cytokine profiles of CD4 positive cells were assessed for intracellular IFN-γ and TNF-α and compared to levels of nonpregnant controls. The percentage of peripheral T cells producing IFN-γ in response to stimulation reduced in pregnancy from 10.7% in nonpregnant women to 6.7% at 28 weeks, 5.1% at term, and 5.6% at term in labour (Sykes, et. al. 2012). Additionally, there was a reduction in the proportion of TNF-α producing cells, although not significantly, from 20.6% in nonpregnant women to 14.5% at 28 weeks, 15.8% at term and 13.3% at term in labour. Overall levels of Th1 cytokine production (expressed as mean fluorescence intensity), in the CD4+/IFN-γ+ or CD4+/TNF-α+ cells, remained consistent throughout gestation. The Th2 cytokine, IL-4, was similarly assessed in CRTH2 positive cells. While PMA/ionomycin stimulation did not increase the percentage of IL-4 expressing cells, the mean fluorescence intensity of IL-4 was significantly increased in samples collected from women at 28 weeks (39.3,) and at term (39.4,) compared to levels of nonpregnant controls (37.1) (see Figure 1). Levels of IL-4 in term labouring samples were consistent with non labouring samples (37.1). The ratio of the IFN-γ:IL-4 producing cells reduces during pregnancy, due to the suppression of the Th1 rather than the promotion of the Th2 cytokine production (Sykes, et. al. 2012).

Many researchers agree that cytokines play a crucial role during pregnancy. Koch and Platt mutually agree that a Th2 response is necessary for the fetus to survive in the womb. Results from an experiment with mice showed that there was a 20-50% rate of fetal loss due to a lack of Th2 cytokine production. Furthermore, they applied this idea to humans and suggested that irregularities with Th2 cytokine response may lead to miscarriages. However, the researchers advise for additional investigation in order to validate the cytokine-shift hypothesis.

Local Active Suppression of Decidua

In addition to the general suppression of a mother's immune system, researchers have found that an important role in the maternofetal interaction is the local active suppression by cells in the decidua (Chaouat, 1990). The decidua is the thick layer of modified mucous membrane which lines the uterus during pregnancy and is shed with the afterbirth. This lining allows for nutrition and gas exchange before the placenta is functional (Mizugishi, et.

al. 2007). Despite its importance for embryogenesis, the development and function of the decidua remains very poorly understood. "On the one hand, we are pursuing the possibility that decidual tissue impairs the overall function of dendritic cells" (Erlebacher, 2010). Critical for initiating T cell-mediated immune responses within lymph nodes, these cells are the most potent antigen-presenting cells. The research team has discovered, in a mouse model, that the onset of pregnancy causes the genes that are responsible for recruiting immune cells to sites of inflammation to be turned off within the decidua. As a result of these changes, T cells are not able to accumulate inside the decidua and therefore do not attack the fetus and placenta. Informatively, they revealed that the implantation of an embryo changes the packaging of certain chemokine genes in the nuclei of the developing decidua's stromal cells. The change in the DNA packaging permanently deactivates, or "silences," the chemokine genes. Consequently, the chemokines are not expressed and T cells are not recruited to the site of embryo implantation (Erlebacher, 2010). Therefore, local active suppression versus systemic suppression aids in the prevention of foreign objects attacking the mother's immune system and fetal rejection (Chaouat, 1990).

Also of note, the observed change in the DNA packaging was an 'epigenetic' modification, meaning a modification that changes gene expression without the presence of a heritable gene mutation. This explains the mechanisms of fetal-maternal immune tolerance as a modality for limiting the trafficking of activated T cells. They concluded that the decidua appears as a zone of relative immunological inactivity due to the fact that the cells that typically secrete the chemoattractants to bring the T cells to sites of inflammation are inhibited from doing so in the context of the pregnant uterus. (Nancy, et. al., 2012). Inappropriate regulation of this process, Dr. Erlebacher explained, could cause inflammation and the accumulation of immune cells at the maternal-fetal interface, which could lead to complications of human pregnancy, including preterm labor, preeclampsia and spontaneous abortion.

While this hypothesis holds the most significance and the least flaws it still needs to be adjusted. A study showed that removing macrophages (an immune cell) caused pregnant mice to miscarry (Mor, 2007). This, along with the antibodies produced, as mentioned above, highlights the immune system activity that exists among the maternofetal relationship.

Conclusion

The phenomenon of a pregnancy and what it entails is astounding. Attempting to understand the intricacies of the maternofetal relationship forces us to realize the vastness of this topic and how much more research is needed to unravel this complex "riddle". With many theories being investigated, it appears that the hypothesis that holds most credence in explaining how a fetus with foreign antigens survives the mothers immune system is the local active suppression within the decidua. Nevertheless, amongst these hypotheses, there is more research that needs to be done concerning the role of the immune system in pregnancy. Pregnancy is complex and is divided into stages. Many researchers believe that labour, which brings the pregnancy to completion, is an immune response. There seems to be the need of a balance between the immune system activity versus inactivity depending on the stage of pregnancy.

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