

Review

How immune mechanisms are affected by pregnancy

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Abstract

Pregnancy requires physiologic adaptations in all maternal systems, including the immune system. This process is complex and includes modifications at different levels and compartments of the maternal immune system. Although many of these changes are only partially explored and understood, recent investigations have showed that during pregnancy maternal circulating immune cells undergo modifications in cell counts, phenotypes, functions and ability to produce soluble factors, such as cytokines. The ultimate goal is to establish and maintain a successful pregnancy, which involves a state of selective immune tolerance, immunosuppression and immunomodulation in the presence of a strong anti-microbial immunity. The mammalian immune system has evolved to coexist with these needs by down-regulating potentially dangerous T-cell-mediated immune responses, while activating certain components of the innate immune system, such as monocytes and neutrophils. This unique dysregulation between different components of the immune system plays a central role in the maternal adaptation to pregnancy.

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The concept that pregnancy is an immunosuppressive state permitting the fetal allograft to implant and grow has held center stage for years. In fact, the placenta is not a cell-impermeable barrier preventing exposure of fetal alloantigens to maternal T-cells, as was suggested by Medawar [1]; rather, maternal and fetal cells are reciprocally transported across the placenta during gestation. Indeed, whole cells of fetal origin [2–4] and fetal DNA [5] have immediate access to maternal venous blood, where they can persist long after delivery [3,5]. These findings indicate that one area of potential maternal–fetal immunological interaction is represented by the maternal peripheral circulation and that mechanisms that block immune responsiveness against fetal alloantigens must take place.

It has been shown that approximately 30% of women develop IgG antibodies against the inherited paternal human leukocyte antigen (HLA) of the fetus along with the presence of cytotoxic T lymphocytes specific for these HLA antigens [6]. The role of these antibodies is not clear and there is no evidence of attack to the fetus during gestation. Maternal T-cell awareness to fetal alloantigens has also been

demonstrated in the pregnant rodent [7–9]. In these models, fetal-specific maternal T-cells are in a state of functional unresponsiveness or are tolerized by deletional or non-deletional processes [7–9]. In humans, lack of maternal immune reactivity to the fetus is thought to be a consequence of a decrease of cytotoxic (CD8⁺) T-cells during pregnancy [10]. To date, the mechanisms by which peripheral tolerance to fetal antigens is achieved and maintained in humans are poorly understood. The current view contemplates that several adaptations may occur in parallel in different compartments of the maternal immune system (Fig. 1).

2. Effect of pregnancy on the numbers of the different subsets of circulating leukocytes

Several studies have focused on the changes in the total number and percentage distribution of the different subsets of maternal peripheral blood leukocytes, often with contradicting results. For example, within the literature, there are reports that pregnancy is associated with a decrease [11], no change [12] or increase [13] in CD8⁺ T-cells. Discrepancies also exist for the CD4⁺ (helper) T-cell subset, as there is evidence of no change [13,14] or decrease [10] during pregnancy. We have found that pregnancy is associated with a significant decrease in the frequency of CD4⁺ T-cells

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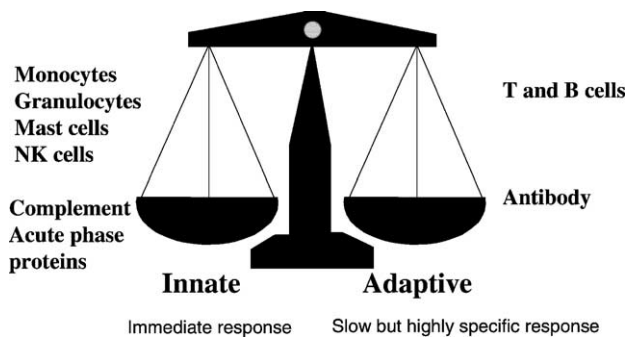


Fig. 1. The two arms of the immune system. The body is composed by two immune systems: the innate and the adaptive immune system, each of them including a cellular and a humoral component. The innate immune system represents the more primitive arm of the immune system and is designed to mount an immediate, antigen-non-specific, response after exposure to an invading microorganism. Innate defense mechanisms are mostly represented by phagocytosis and killing of the microorganism. Recognition of an antigen by the innate immune system is mediated by pattern-recognition receptors, which recognize highly conserved structures present in many different microorganisms. The innate immune system involves phagocytic cells (neutrophils and monocytes/macrophages), mast-cells, natural killer cells and humoral proteins, such as complement and acute phase proteins. The adaptive immune response, instead, refers to antigen-specific defense mechanisms that take several days to become protective as they are designed to remove a specific antigen. It involves a specific process of antigen recognition mediated by high affinity receptors localized on T and B-cells leading to clonal expansion of cells with high affinity, very specific antigen-receptors and antibodies. The effect of pregnancy is on both the innate and the adaptive maternal immune systems.

and with an increase in CD8⁺ T-cells, the latter especially during labor [15]. Overall, there is no uniformity between these studies to show that there is a consistent deviation in the number or percentage of T-cells and/or T-cell subpop-

ulations during pregnancy. Frequency and counts of B-cells also appear to be undeviated during pregnancy [10,13,15]. Absolute numbers of natural killer (NK) cells increases in early pregnancy and decreases in late pregnancy [10,13].

One of the most substantial immunologic deviations occurring in pregnancy is an increase in the proportion and absolute numbers of granulocytes [15]. By following pregnant women longitudinally during gestation, we found that this increase was evident as early as 3 weeks of gestation, reaching significance at 13–28 weeks as compared to non-pregnant women [16] (Fig. 2). After this stage, the proportion of granulocytes remained significantly higher compared to non-pregnant women. The percentage of monocytes did not show any significant changes throughout pregnancy and was similar to non-pregnant controls (see Fig. 2).

3. Effect of pregnancy on the phenotype of circulating immune cells

Our laboratory [16], together with Sacks et al. [17], has shown that normal pregnancy is characterized by a generalized activation of circulating granulocytes and monocytes. This result was demonstrated by the presence of modifications in surface expression of adhesion molecules (increase in CD11a, CD11b, CD54 and decrease in CD62L), which are molecules playing an important role in mediating leukocyte responses to inflammatory stimuli and cell–cell or cell–matrix interactions. The most marked modifications in terms of number and degree occurred in monocytes. These latter cells also showed an increased expression of the high affinity receptor for the Fc portion of the immunoglobulin G (FcγR-I, CD64), which mediates phagocytosis, intracellular

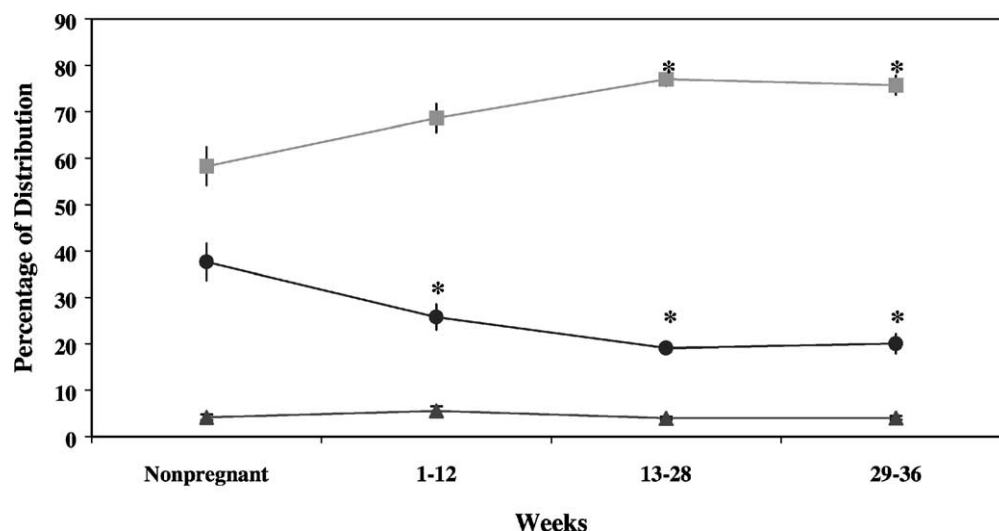


Fig. 2. Percentage distribution of circulating leukocytes throughout pregnancy. Shown is the proportion of lymphocytes (●), granulocytes (■), and monocytes (▲) in peripheral blood of women in the non-pregnant state, at 1–12, 13–28 and 29–36 weeks of gestation as determined by flow cytometry. Data are expressed as mean percentage $\pm$ S.E.M. in the different groups. The percentage distribution of the different subsets of leukocytes was calculated by setting gates around the different circulating elements based on their side-light-scatter (SSC) and forward-light-scatter (FSC) characteristics, as described [16]. Statistically significant differences for each leukocyte population between non-pregnant and pregnant women are marked with an *.

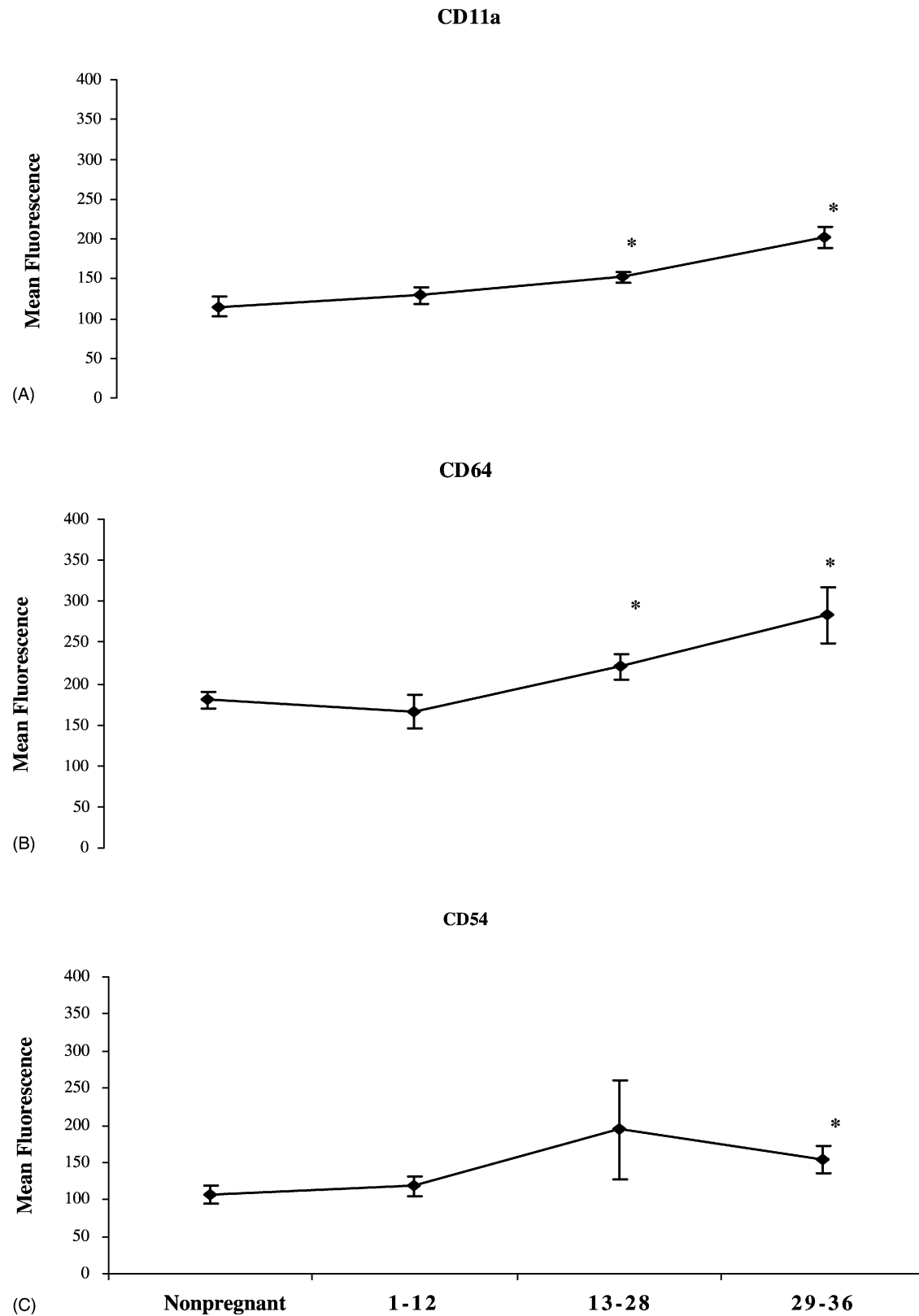


Fig. 3. Increased expression of CD11a (A), CD64 (B), and CD54 antigens (C) on monocytes during pregnancy. The results show changes in mean fluorescence for CD11a (A), CD64 (B), and CD54 antigen (C) on monocytes in the non-pregnant state, at 1–12, 13–28 and 29–36 weeks of gestation. The analysis of the expression of the different surface markers on monocytes (CD14⁺) was performed by setting specific gates for the monocyte population based on dot-plot graphs of SSC vs. CD14⁺ cells, a monocyte specific marker, as described [16]. Data are expressed as geometric mean fluorescence values $\pm$ S.E.M. for each marker in the different groups of women. Statistically significant differences for each surface marker between non-pregnant and pregnant women are marked with an *.

killing of microorganisms, and display accessory and immunoregulatory activities to T-cells [16]. Data on pregnant women followed longitudinally at different time-points during gestation showed a progressive increase in monocyte activation as pregnancy progressed, as evidenced by significant up-regulation of the CD11a, CD54 and CD64 antigens, reaching the highest level of expression at 29–36 weeks of gestation (16) (Fig. 3). A further increase of adhesion molecule expression was observed during labour at term (Luppi et al., unpublished data). Additional evidence of monocyte activation during pregnancy is the progressive increase in plasma concentration of one cytokine of the TGF- β family, called macrophage inhibitory cytokine-1 (MIC-1) [18] and of neopterin, a product of the guanosine pathway in monocytes [19]. These molecules are considered markers of monocyte activation. Moreover, there also appears to be activation of the transcription factor NF- κ B at the level of DNA binding (Luppi, unpublished data). Conversely, T lymphocytes do not show any significant evidence of modification in the expression of surface antigens throughout pregnancy [16].

4. Effect of pregnancy on the functions of circulating leukocytes

4.1. T- and B-cell functions

The majority of studies on immune cell function during pregnancy have focused on T lymphocytes, and have demonstrated an impairment of proliferation and of IL-2 and IFN- γ secretion after *in vitro* stimulation with either concanavalin A (ConA), phytoemagglutinin (PHA) or foreign antigens as compared to the non-pregnant state [14,20,21]. When T-cells are stimulated with phorbol-myristate-acetate (PMA), which directly activates protein kinase C, a significant decrease in the potential of synthesizing IL-6 was also detected [16]. Suppression of T-cell activity during pregnancy has been suggested to be at the basis of the increased susceptibility to viral infections [21,22] and to specific intracellular pathogens, such as *Listeria*, that also survive phagocytosis by monocytes [23]. Further evidence supporting T-cell suppression during pregnancy is the remission of rheumatoid arthritis and multiple sclerosis, two T-cell-mediated autoimmune disorders, in the majority of pregnant women who have arthritis or suffer from multiple sclerosis [24,25]. Conversely, B-cell function and antibody production to vaccinations are normal during pregnancy [22]. This suggests that the maternal immune system, when activated, is more likely to respond with antibody and less likely to respond with activated T-cells. This increased B-cell activity is thought to be responsible for the greater incidence of flare-ups of systemic lupus erythematosus during pregnancy [26].

A number of factors have been proposed to explain the effects of pregnancy on T- and B-cell functions. Hormones, such as estrogen and progesterone, modulate T-cell reactiv-

ity and production of cytokines [27]. Normal pregnancy has long been described as a state characterized by the presence of a type 2 immune response bias, with preferential production of IL-4, IL-5 and IL-10, cytokines that strengthen humoral immunity. Type 1 cytokines, such as IFN- γ , tumor necrosis factor (TNF)- α and IL-2 are, on the contrary, down-regulated. This shift from a type 1 to a type 2 reactivity would lead to suppression of cell-mediated immune responses that are potentially detrimental to the fetus [27,28]. And, in fact, several groups have shown that in both rodent models and humans, disruption of the cytokine balance, at least at the maternal–fetal interface, can lead to activated leukocytes [28,29] and causes pregnancy failure [30]. While there is little doubt that there can be down-regulation, deletion or anergy of T-cells in the pregnant rodent [7–9], new data shed doubt on the type 1/type 2 immune response paradigm by demonstrating how IFN- γ but not IL-4 and IL-10 is crucial for a successful pregnancy. In fact, breeding experiments with IL-4 and IL-10 double-deficient mice have indicated that neither maternal nor feto-placental deficiency of these cytokines is crucial for fetal or neonatal survival [31]. IFN- γ , instead, appears to be essential to initiate the pregnancy-induced remodeling of decidual arteries permitting vessel wall thinning and lumen increase [32]. Moreover, IFN- γ is a critical factor in conferring protection against placental malaria, a condition characterized by accumulation of malarial parasites in the intervillous spaces of the placenta and leading to intrauterine growth retardation and low birth weight in the infant [33,34].

Overall, these data suggest that modulation of maternal T-cell responsiveness is a complex phenomenon and numerous adaptations could operate in parallel to generate and maintain a state of immunosuppression. Recently, the presence of immunosuppressive factors in the serum of pregnant women has been demonstrated [20,25]. One of these factors is represented by the pregnancy-specific glycoprotein-1a (PSG1a), which is synthesized in large amounts by placental trophoblasts and is able to function as an important T-cell modulator agent at the systemic level [35]. Another factor with immunosuppressive properties is prostaglandin E₂ (PGE₂). PGE₂ is a potent suppressor of both monocyte antigen presenting function and T-cell expression of IL-2.

4.2. Monocytes, granulocytes and other components of the innate immune system

Granulocytes and monocytes play a fundamental role in the host's response to infection through the phagocytosis and the killing of microorganisms. When activated, these cells produce a series of products derived from the reduction of molecular oxygen. These products are known collectively as reactive oxygen species (ROS), and are required for microbicidal purposes. While there is general agreement on the increased ROS production and enhanced phagocytosis by monocytes [17], published data on pregnancy granulocytes remain conflicting. Sacks et al. reported enhanced

phagocytosis as compared to non-pregnant women [17]. However, two other groups, working independently, reported depressed granulocyte function, with reduced killing of bacteria and chemotaxis [36,37], as well as decreased respiratory burst activity and intracellular H_2O_2 production when challenged [38,39]. Further studies are required to clarify this controversy. We found that in late gestation granulocytes have a greater ability to synthesize the cytokine IL-8 as compared to non-pregnant women [16]. Granulocytes are not only producers, but also the primary targets for IL-8, responding to this mediator by chemotaxis, release of granule enzymes, respiratory burst activity, up-regulation of adhesion molecules and increased adherence to endothelial cells.

Monocytes from pregnant women also show a greater ability to produce the pro-inflammatory cytokines IL-12 and IL-1 β as compared to the non-pregnant state [16]. This increase was significant from 13 weeks of gestation onward. However, the potential to synthesize TNF- α was decreased, suggesting tight regulation in the production of inflammatory cytokines. Hormones, such as progesterone and estradiol may modulate cytokine synthesis during pregnancy. These findings suggest concordance of monocyte phenotype with production of pro-inflammatory cytokines [16].

Not all components of the innate immune system are activated in the maternal circulation. For example, cytotoxic activity and IFN- γ production by NK cells are suppressed [40]. However, these cells represent the major leukocyte population (~90%) at the maternal–fetal interface in the deciduas [2]. Because these cells provide protection from viruses and perhaps from bacteria and parasites as well, their presence at the fetal–maternal interface may prevent pathogens from going to the fetus. In addition, they may promote fetal growth by secretion of cytokines.

Another component of the innate immune system that shows modifications during pregnancy is the complement system. This system is a protein cascade that circulates in the bloodstream and culminates in the synthesis of powerful lytic complexes for the destruction of infectious agents. Beginning at 11 weeks of gestation, maternal serum complement elevations occur that enhance chemotaxis and Ig opsonization to improve maternal defense against bacterial invasion [40].

5. Conclusions

During a normal pregnancy, modifications occur in virtually every facet of the immune response. Enhancement occurs in the numbers and activation state of circulating granulocytes and monocytes, leading to more aggressive attack on invading bacteria. T-cell and NK cell functions depression and reduced secretion of type 1 cytokines protect the fetus from destruction by maternal immune responses. Thus, a general balance between enhancement and suppression is achieved, leaving maternal defenses intact (Fig. 4). Monocytes, with their antigen presenting function and im-

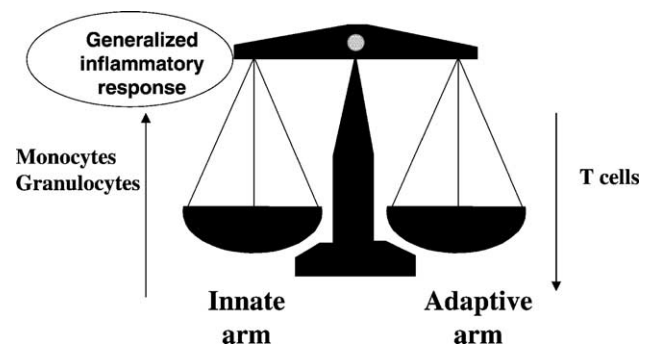


Fig. 4. Pregnancy is associated with a generalized inflammatory response. In pregnancy, the innate immune system is activated, and the adaptive immune system is suppressed. Monocytes and granulocytes appear to be the cells most profoundly affected during gestation as shown by several changes in phenotypes and functions. Conversely, T-cells functions appear to be down-regulated during gestation. This unique dysregulation between the innate and the adaptive arms of the immune system is thought to be crucial for the maintenance of a successful pregnancy [40].

munomodulatory capability, appear to be key elements in maternal homeostasis during pregnancy. These cells, besides their role in host's defense, can participate in the maintenance of peripheral tolerance to fetal alloantigens by displaying tolerogenic signals to T-cells. This can be achieved through modifications in surface expression of antigens and co-stimulatory molecules, secretion of soluble factors with immunosuppressive activity [41] and cytokines, or metabolic effects (i.e. degradation of tryptophan by enhanced activity of the indoleamine dioxygenase enzyme) [42,43].

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