

NK Cells, Autoantibodies, and Immunologic Infertility: A Complex Interplay

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Abstract Infertility and recurrent spontaneous abortion (RSA) are heterogeneous conditions that have been frequently explained with an immunological pathomechanism. A deeper insight into apparently unexplained infertility and RSA shows increasing evidences supporting both alloimmune and autoimmune mechanisms, in which natural killer (NK) cells and autoantibodies seem to play a relevant role. Successful pregnancy is considered as Th1–Th2 cooperation phenomenon, with a predominantly Th2-type lymphocytes response, together with the emerging role of interleukin (IL)-12, IL-15, and IL-18 and of other unidentified soluble factors dependent on NK cells. Uterine NK cells comprise the largest population at implantation site, and their activity, characteristics, and abundance suggest that they participate at the “decidualization” process that, vice versa, induces NK activation and recruitment in each menstrual cycle. However, NK cell alteration may be associated with impaired pregnancy, and the modulation in the number of circulating NK cells is most likely to be a

primary event rather than an active inflammation/drug administration consequence during an inflammatory/auto-immune process, thus playing an important role in the pathogenesis of immunological infertility. Relationships within immunological infertility, recurrent spontaneous abortion, autoantibodies, and NK cells will be reviewed herein.

Keywords NK cells · Autoantibodies · Immunologic infertility · Recurrent spontaneous abortion · APS

Introduction

Infertility and reproduction

Infertility, the biological inability of a person to contribute to pregnancy and more in general, of a couple to conceive, and recurrent spontaneous abortion (RSA), a heterogeneous condition often idiopathic, defined by three or more consecutive spontaneous abortions, that has numerous causes and clinical presentations and may occur during any stage of pregnancy, have been frequently explained with an immunological pathomechanism [1, 2]. A deeper insight into apparently unexplained infertility and RSA shows increasing evidences supporting both alloimmune and autoimmune mechanisms, in which natural killer (NK) cells and autoantibodies seem to play a relevant role [3].

Pregnancy and the maternal–fetal interface

Implantation is an event depending on at least several steps. At every step, there is a continuous embryo–uterus interaction, and as the implantation window was discovered

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and the concept of uterine receptivity emerged, the idea that cytokines could be central in such a process was dramatically raised [4] (Fig. 1). In human placentation, fetus-derived extravillous trophoblast infiltrates the decidua and replaces the endothelium of the uterine spiral arteries to ensure the development of an adequate blood supply to the fetal–maternal unit [5].

During apposition/adhesion, the induction of adhesion molecules and the proper ligands are critical steps. Hence, it is very important that the expression of receptors/ligands at the cell surface of the embryo and uterus coincides. There is an involvement of interleukins, but also an important hormonal regulation, both in the uterus and on the embryo. Adhesion molecules are also modulated. Subsequently, invasion–penetration occurs, and at this step, several enzymes are involved, especially matrix metalloproteases, counterbalanced by their inhibitors.

Successful pregnancy is considered as T helper (Th)1–Th2 cooperation phenomenon, with a predominantly Th2-type lymphocytes response, together with the emerging role of interleukin (IL)-12, IL-15, and IL-18 and of other unidentified soluble factors dependent on NK cells [6]. This immunological response against trophoblast antigens, concomitant with a transient depression of maternal cell-mediated immunity to protect the semiallogenic embryo from rejection, is the predominant mechanism for high live birth rate.

Th1/Th2 paradigm would predict that inflammatory molecules are deleterious for implantation. The release of Th1 cytokines such as tumor necrosis factor (TNF)- α and interferon (IFN)- γ is associated with increased decidual and trophoblast cell apoptosis, possibly through an alteration of the bcl2:bax ratio [7].

Wegmann first introduced the idea that the maternal–fetal relationship was bidirectional and that immunostimulation might be more important than immunosuppression [8]. His group also suggested that allopregnancy is a Th2

phenomenon, the Th1 cytokines being abortifacient and the Th2 cytokines being protective. The main limit of the Th1/Th2 model in pregnancy is the exclusion of NK cells as having the central role and does not predict the missing self concept nor the NK-mediated immune control of vascularization. Furthermore, the generic distinction within proinflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines cannot be considered as conclusive. Uterine NK cells (UNKs) comprise the largest population at implantation site, and their activity, characteristics, and abundance suggest that they participate at the “decidualization” process that, vice versa, induces NK activation and recruitment in each menstrual cycle [9].

The Th1/Th2 concept has been even extended by demonstrating that NK cells also can be differentiated by comparable polarities in their cytokine secretion profiles [10, 11]. Two main populations have been suggested, NK1 and NK2. Peripheral NK cells (PNKs) cytokine repertoire comprises mainly type 1 cytokines, such as IFN- γ and TNF- α , although proper stimuli can induce type 2 cytokines such as IL-4, IL-5, and IL-13 production [12–15].

PNK cells

Cantor, Wigzell, Playfair, and other authors described between 1974 and 1977 a novel lymphocyte effector function mediated by cells that were large granulocytic lymphocytes, yet were considered neither T cells nor B cells and did not use related families of antigen receptors. These cells, called large granular lymphocytes or better known as NK cells, are part of the innate immune system and respond rapidly to a variety of insults via cytokine secretion and cytolytic activity [16–19]. The main role of these critical components of the innate immune system is antiviral immune response, and the effector function is perceived to be an important first line of innate immunity across viral, bacterial, and parasitic infections [20]. PNK cells that are thymus-independent and do not require preimmunization thus reflecting their innate character express the activation markers CD16, CD56, and CD57, the inhibitory marker CD158a, and CD94, which can be either inhibitory or activatory [21, 22]. PNK cells are controlled by many activating and inhibitory receptors. Indeed, they have a very sophisticated program for control of activation and killing which is now understood to be a highly complex system of diverse, inhibitory, and activatory receptor–ligand interactions, sensing change in human leukocyte antigen (HLA) expression. Recent data have shown that PNK cells in addition to direct cytotoxic and antiviral functions are not solely killers but can also act as regulators of adaptive immunity, for example, by interacting and providing stimulatory signals for the components of the adaptive immune system, including T cells and dendritic

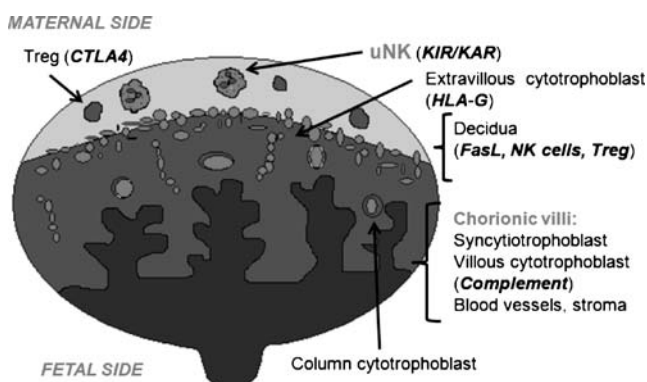


Fig. 1 The maternal–fetal interface: role of uterine NK cells

cells [23–25]. This interaction implies a broad role in immunity and potential involvement in a wide range of diseases including infections, cancers, and especially, autoimmune disorders.

PNK cells involvement in the regulation of inflammatory and autoimmune responses (loss of self-tolerance at multiple steps) is well established and generally accepted, and the paradigm that innate immunity plays an instructive role in the adaptive immune response is well established as well [26]. Recent studies suggest that PNK cells may play distinct roles during different forms and at different stages of immune and autoimmune responses. Thus, it has been suggested that PNK cells may either prevent/limit autoimmune responses or may have a permissive role in autoimmunity [3].

PNK cells play an essential role as surveillance cells for tumorigenic, virus-infected, and allogeneic cells and recognize targets by the absent or aberrant expression of self-major histocompatibility complex I molecules. The right balance among signals received by the PNK cells and activating or inhibitory ligands will determine the fate of the target cell. Allogeneic cells can be introduced via insemination or during implantation and placental formation, thus regulation of NK cell activity is crucial. In addition, PNK cells play a role in successful pregnancies by providing angiogenic signals required for proper placental formation and spiral artery maturation [27].

NK cells constitute the predominant leukocyte population present in the endometrium at the time of implantation and in early pregnancy while normally comprise about 10–15% of peripheral blood lymphocytes. During normal pregnancy, peripheral NK cell activity and percentages tend to increase in the first trimester and then tend to decline from the second trimester with a second fall in the third trimester of pregnancy [28].

UNK cells

UNK cells are classically CD56⁺/CD16[–] but can readily be activated by short-term exposure to cytokines such as IL-2 to become lymphokine-activated killer cells that express CD16 and release TNF- α and IFN- γ , Th1 cytokines. UNK cells can produce also granulocyte macrophage-colony stimulating factor (GM-CSF), IL-10, TGF- β , IL-8, and leukaemia inhibitory factor, the latter which is required for implantation in mice and differentiate upon IL-15 release via the endometrium [29]. Similar to peripheral NK cells, UNK cells express CD94 and killer cell immunoglobulin-like receptor(s) (KIR(s)) but several genes as HLA-E receptor differ in expression between blood peripheral and UNK cells. Indeed, the UNK cells are predominantly CD56^{bright} as they do not express CD16 or membrane CD3 and have a lower cytolytic activity against K562 cells

respect to granulated CD56^{dim} CD16^{bright} peripheral NK cells and in turn, an overall reduced cytotoxic activity [30, 31]. UNK cells are important for establishing spiral artery formation in the endometrium by releasing IFN- γ [32, 33]. It was demonstrated that IFN- γ -deficient mice exhibit pre-eclampsia-like symptoms and results in lower litter size [32]. In the nonpregnant endometrium, the number of UNK cells varies with the menstrual cycle, being low in the follicular phase, increasing after ovulation, and reaching a peak in the late luteal phase [34]. Finally, following the decrease in progesterone levels and before the menstrual shedding of the endometrium, UNK cells undergo extensive apoptosis. However, if pregnancy occurs, UNK cells will expand at the implantation site until midgestation, and in early pregnancy, UNK cells accumulate as a dense infiltrate around the trophoblast spiral arteries [35]. The placenta and endometrium are thought to attract UNK cells via CCL3 [36]. UNK cells then will gradually disappear, being virtually absent in term deciduas, suggesting that these cells are only needed for the initial vascularization of the placenta.

UNK cells during menstrual cycle account for almost the 70% of total leukocytes prior to menstruation during late secretory phase. After implantation, the leukocytes increase from less than 10% of the stromal cells as they are in the proliferative and early secretory phase endometrium to over 30% due to the increase in UNK [37].

Available data support that pro-UNK cells are widely distributed in the periphery, then, during the early stages of normal pregnancy, significantly proliferate in situ, under the influence of stroma-derived IL-15 and prolactin, that are required for full differentiation during the process of decidualization [38]. The expanded uterine NK population then plays an important role through cytokine production in supporting proper trophoblast and placental growth as well as vascularization of the decidua. In addition, through production of immunomodulatory molecules, they may participate in the creation of local immunosuppression at the maternal–fetal interface. These are most pronounced during the phase of trophoblast invasion into the stroma with redundancy in epithelial immunosuppressant, e.g., glycodefin, during blastocyst attachment, and intrusion into the endometrium [39].

It has been showed that UNK cells have a different phenotype and activities than peripheral NK cells. The number and activities of the peripheral cells are poorly representative of local UNK, also because regulatory molecules and receptors localized in peripheral blood lymphocytes are not the same and whether present may not work properly in the uterus. PNK and UNK can be activated in many ways, and high local numbers are physiological during the peri-implantation period when they secrete IL-18 [40].

NK cells: a protective role in pregnancy?

It has been proposed that cytokine production by NK cells facilitates decidualization, controls trophoblast invasion, and promotes angiogenesis at the implantation site [32, 41].

Several evidences showed that PNK cells have a positive role in implantation, for example, by controlling the IL-10 secretion by the placenta as a consequence of the alloimmunization process [42]. Nonetheless, they can secrete IL-10 by themselves if properly stimulated. One of the main evidences in favor of a protective role of NK cells in pregnancy comes from animal models showing NK-knock-out mice undergoing important peri-implantation defects and postimplantation defects related to the vascular bed [43].

As aforementioned, the UNK play a role in placental vasculature remodeling. One of the genes most upregulated in UNK encodes galectin-1 (gal1) that induces apoptosis of activated CD8 T cells and induces Th2 cytokine shifts. Various immunosuppressive mechanisms have been proposed to protect the fetus from potentially alloreactive T cells. A novel mechanism may involve the induction of apoptosis of decidual T cells in the human placenta mediated by gal1, produced by UNK [44].

Other evidences showed that cytokine secretion by resting human NK cells is induced by soluble, but not solid phase, antibodies to the KIR 2DL4, a receptor for HLA-G. The profile of genes upregulated by KIR2DL4 engagement on resting NK cells revealed a proinflammatory/proangiogenic response. Soluble HLA-G, which is endocytosed by KIR2DL4, may stimulate resting NK cells and implies that NK cells may provide useful functions at sites of HLA-G expression, such promoting of vascularization in maternal decidua during early pregnancy [45]. Macrophage migration inhibitory factor (MIF), the first cytokine to be discovered, is now recognized as an inhibitor of the random migration of macrophages *in vitro* and can affect several biological functions of macrophages such as phagocytosis and killing of intracellular parasites and tumor cells exerting both an autocrine and a paracrine modulatory action on UNK cell activity. MIF may contribute to the immune privilege at the maternal–fetal interface [46].

Finally, regulatory NK cells may play essential roles in the maintenance of pregnancy as both allogeneic pregnant nonobese diabetic mice that show significantly high percentage of spontaneous embryo loss demonstrate a further increase in pregnancy loss by the administration of anti-asialo ganglio-*N*-tetraosylceramide to deplete NK cells, and decreased by the adoptive transfer of CD49b⁺ CD25⁺ NK cells from normal mice. Recruitment of peripheral CXCR4-expressing CD49b⁺ CD25⁺ NK cells into pregnant uteri may be again important in the regulation of fetal–maternal tolerance [47].

NK cells: harmful in pregnancy?

However, the number and status of NK cells, whether activated or inhibited, appears to be crucial in the success of pregnancies, and populations in endometrium and circulation may differ, contributing to unexplained infertility. For instance, it has been suggested that, in infertile women, changes in circulating NK cell percentages are found exclusively during the secretory phase and not in endometrium. Specifically, increased expression of CD94, CD158b (secretory phase), and CD158a (proliferative phase) by endometrial NK cells from infertile women has been described [48].

Even more intriguing, NK cells have long been suspected to cause RSA since Aoki's original report [49]. In RSA, increased numbers and killing activity of NK cells in the peripheral blood predict the likelihood of another miscarriage and are considered as a causal and prognostic factor for sterility, infertility, and miscarriage. Thus, NK cells may also play an important role in immunological infertility and in RSA when in too high concentrations [50]. In fact, high concentrations of NK cells of the conventional CD56⁺ 16[−] type have been found in the uterus of women who have abortions suggesting that the cytotoxic activity is probably present at the implantation site. Several studies showed that women with RSA have higher proportion and activity of NK cells, which could reflect the production of a cytotoxic response against the fetus. In RSA, creation of an imbalance in Th1–Th2 response, resulting in a prevalent Th1 cytokine environment in the periphery may lead to NK cell activation and proliferation, which could result in migration of cytotoxic NK cells into the uterus and in turn, contribute to mechanisms involved in miscarriage [51]. Alternatively, the local endometrial immune assessment may be disrupted at a variety of levels, causing defects in homing of the proper NK population to the uterus, local production of cytokines and hormones such as IL-15 and prolactin, and impairment of more downstream events such as production of immunoregulatory factors by uterine NK cells. Disruption at any of these levels may alter the ability of the uterine NK cells population to perform its normal functions and may result in an unsuccessful pregnancy [52].

Considering the Th1/Th2 paradigm, failure in the generation of Th2-type responses and consequent imbalance in Th1–Th2 response are associated with recurrent abortions, obstetric complications, and poor pregnancy outcome. In peripheral blood, NK cells are either CD56^{bright} or CD56^{dim} cells, the main population being CD56^{dim} NK cells. CD56^{bright} cells are also present in the circulation, but high levels are in the decidua. It has been suggested that the type 2 shift during pregnancy is predominantly in the NK cell (CD56^{bright} and CD56^{dim}) and NKT cell (CD56⁺CD3⁺) populations instead of in the T helper or cytotoxic T cell

populations [53, 54]. It has been shown that women with RSA and implantation failures show higher proportion (percentage) of CD56^{bright}/interferon- γ ⁺/TNF- α ⁺ cells compared with healthy controls and lesser of CD56^{bright}/IL-4⁺/IL-10⁺ cells although very low (<2%) in all groups. It has been suggested that, in nonpregnant women with RSA and implantation failures, there might be an overproduction of these cytokines, especially TNF- α that may be one of the causes of the reproductive failures [55].

Another recent study identified a population composed both of NK cells together with T cells to be responsible for a predominant Th1 immunity against trophoblast [56]. It has been suggested that cell-mediated (Th1) immunity toward trophoblast cells is mostly suppressed in normal pregnancy, and that, perhaps, failure of such suppression contributes to loss of the pregnancy in case of unexplained RSA. An impaired Th1 response may lead into NK cell activation and proliferation, which could result in migration of cytotoxic NK cells into the uterus and in turn, contribute to mechanisms involved in miscarriage.

Cytokine imbalances are particularly relevant as cytokines may affect the activation or inhibition of NK cells and as the balance of proinflammatory and anti-inflammatory cytokines may be altered in RSA. Disruption at any of these levels may alter the ability of the uterine NK cells population to perform its normal functions and may result in an unsuccessful pregnancy (Table 1).

Given the nature of IFN- γ as a damage mediator and the skill of NK cells to kill other cells, it seems that NK cells may rather have a disease-promoting role in pregnancy. The interaction with the adaptive immune system could facilitate both the priming phase and the effector phase of the immune response with NK cells. IL-21 also might act by stimulating NK cells to become disease-promoting, possibly by stimulating their IFN- γ secretion [57, 58].

Some women with RSA manifest an abnormal cellular immune response with a marked increase in peripheral blood. Considering animal experiments, a murine model of abortion, specifically the CBA-DBA/2, seems to be NK-mediated, and uterine NK cells appear to be crucial [59]. In humans, one of the key receptor families contributing to the NK cell receptor repertoire are the inhibitory and activatory killer cell immunoglobulin (Ig)-like receptor (KIR and KAR) families. Similar to many NK cell receptors, these are expressed on T cells in addition to NK cells, affirming their role in adaptive immunity. They are distinct from many other NK cell receptors, however, in that they are both diverse and rapidly evolving. It might be expected that the existence of KIR or KAR/HLA genotypes that tune NK cells in favor of inhibitory or activatory interactions are advantageous in some infectious disease settings but might tend in some cases to predispose to cytotoxicity [3, 60].

It is very recent the evidence that exists a shift toward the over activation of NK cells based on the individual's KIR gene content in patients with recurrent miscarriages, comprising of a greater number of activating and reduced number of inhibitory KIR2DL1 genes. RSA patients seem to show a higher prevalence of B haplotypes when compared with A haplotypes, and individuals with BB genotypes might be at a greater risk for spontaneous miscarriages than those having AA or AB genotypes [61, 62].

NK cells and autoantibodies

Antiphospholipid antibodies (APL) are found in approximately 5–17% of general population, but are more frequent in patients with unexplained recurrent miscarriage, pelvic diseases (e.g., endometriosis), unexplained infertility, and repeated in vitro fertilization (IVF) failure [63]. The deep relationships within NK cells, autoantibodies, and pregnancy are determinant during a clinical condition which is antiphospholipid syndrome (APS). APS is an autoimmune disease characterized by the presence of one or more laboratory findings of antiphospholipid antibodies and at least one clinical manifestation besides deep venous and/or arterial thrombosis and/or pregnancy disease comprising RSA, with or without thrombocytopenia [64–67]. The association between APS and RSA is well known, so that RSA represents one of the clinical diagnostic criteria for APS. The risk of pregnancy loss in women with APS is higher from the tenth gestational week (GW) onward (fetal period). The Sapporo criteria and the revised criteria for APS underline this situation by considering only patients with one or more unexplained fetal losses (of a morphologically normal fetus) beyond the tenth GW, or three or more unexplained consecutive spontaneous abortions before the tenth GW. As a number of controversies plague the current understanding of APS and RSA, several authors have reviewed the role of APS and specifically of APL in RSA, and they have underlined the existence of different subclasses of clinical subsets of RSA in APS patients [28, 68, 69]. Indeed, it is intrinsic to APS definition that there is a dichotomy regarding patients who have multiple (>3) abortions within the first ten GWs and those who have at least one abortion beyond the tenth GW. An important evidence of as to how NK cells may behave as pathogenic effectors in RSA comes from a recent study that showed NK cells possibly playing a role in the pathogenesis of abortive events in a subpopulation of APS–RSA patients that previously has been explained in terms of autoimmune specific reactions (APL-mediated). In this paper, NK cells were found elevated both in absolute number and in percentage in a subpopulation of APS–RSA patients that

Table 1 Roles of NK cells in pregnancy

Possible roles of NK cells in pregnancy

1. Physiologic

Immunosurveillance

Angiogenesis (necessary for continuation of the pregnancy)

Remodeling of the spiral arteries to uteroplacental arteries

Supporting proper trophoblast and placental growth

Vascularization of the deciduas

Production of immunomodulatory molecules (they may participate in the creation of local immunosuppression at the maternal–fetal interface)

2. Pathologic

Killing–cytolytic activity (due to molecular mimicry between phospholipids, b2-glycoprotein 1, and infectious organisms?)

Inhibiting placental human chorionic gonadotrophin secretion

Complement activation

Cytokine imbalance

Failure in the generation of Th2-type responses

Activate APCs leading to the development of pathogenic Th1 responses

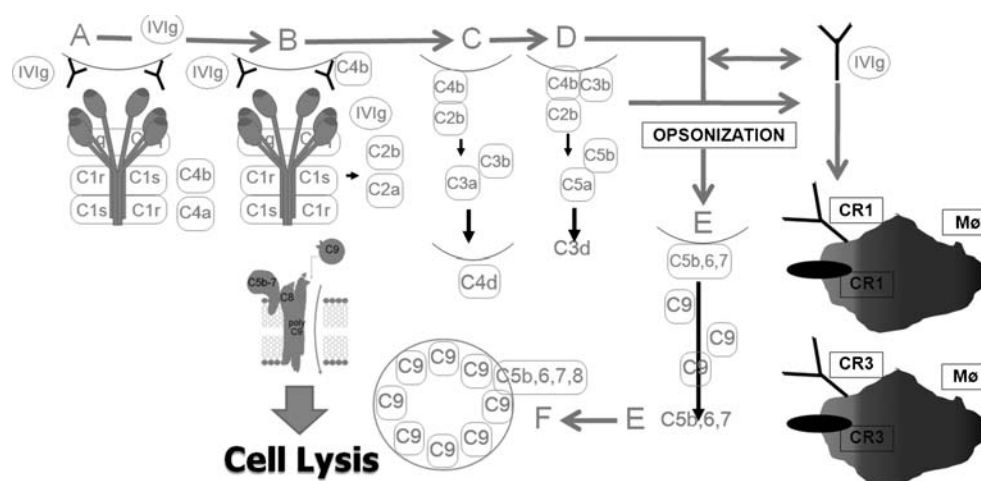
shows specific features in terms of high NK cells levels ($N > 15\%$) and early abortion (within the tenth GW). Interestingly, NK cells levels were elevated not only when compared with normal healthy controls, but also when compared with other RSA populations (except idiopathic), or with another APS population not presenting RSA, and overall, they were higher than the APS–RSA subpopulation showing late abortions (after the tenth GW) [28]. These evidences support the hypothesis that the finding of a high number of circulating NK cells could be a not specific but important peculiarity of tendency to miscarriage [52]: in these patients, the high NK value may not be linked to the condition of being pregnant, but present yet before, as a distinctive mark of their immunity system and may cause damage perhaps due to their ability to provide an early source of cytokines and to activate APCs leading to the development of pathogenic Th1 responses. Whether it is the clue of NK cells in pregnancy, it is evident that, under certain conditions, they may switch from their natural protective role and become aggressive and damaging even leading into (early) abortion [70]. Regardless of the presence of the antiphospholipid syndrome, Beer et al. observed in the 1996 that RSA women and those who had APL showed higher percentages of CD56+ and CD56+/CD16+ cells [71]. However, in patients who repeatedly failed IVF even if showed with higher frequency, an altered immunological profile in terms of presence of autoantibodies (antidouble-stranded DNA (dsDNA), antinuclear, antisperm), and higher levels of NK cells, the two features were not linked, appearing only in one patient concomitantly high NK cells and antisperm antibodies [72].

Not only during RSA but also during IVF failure, APL have been found in higher frequency, and it appeared that antiphosphatidylethanolamine (APE) and antiphosphatidylserine (APS) may have a crucial role. It has been demonstrated that APE and APS positivity in female infertility was associated with increased NK cell activity and decreased IVF success, which did not happen if the APE/APS were found in isolated male factor infertility. Thus, again in this study, APL were considered not as causally related to reproductive failure, rather as markers or intermediaries of an underlying abnormal cellular immunity [73].

NK cells and high doses intravenous immunoglobulins

In RSA, several different therapies have been proposed and within these are high doses intravenous immunoglobulins (IVIG) for refractory cases [74–80]. Immunomodulatory effects of IVIG still remain an enigma [81], although several have been already reported, and among others, antigen neutralization, Fc-receptor blockade and anti-idiotypic interactions, changes in distribution and function of T-cell subsets, antibodies to autoantigens, and mild activation of complement while GM-CSF blood concentration significantly increases after IVIG infusion [82–85] (Fig. 2).

Furthermore, it is known that IVIGs affect cytokine production in T lymphocytes and monocytes/macrophages. Many of these actions may potentially tend to decrease NK cell population and cytotoxicity to divert T cell responses from a prevalent Th1 pattern toward a Th2 one, reassessing the normal cytokine pattern, and to decrease autoantibody titers: all mechanisms of potential benefit in RSA. In a previous study, it has been showed that treatment by means of IVIG during RSA patients pregnancy can revert and lower these high levels to values comparable with those of healthy women. The efficacy of IVIG treatment was confirmed by the evidence of a negative modulation on NK levels even in a very short time, i.e., 30 min after a single infusion [28]. IVIG may exert a chronic and long-lasting beneficial effect justified by its immunoregulatory properties, which include elimination of autoantibodies by increasing the catabolism of immunoglobulins. This mechanism also acts in patients with antiphospholipid antibodies where IVIG work as anti-anti-PL [86–88]. And IVIG also reduce NK cell activity and cytotoxicity in vitro, as recently demonstrated both via CD200-dependent and non-CD200-dependent pathways [89]. This effect has been compared with prednisolone that was almost equally effective in vitro in suppressing NK cell cytolytic activity [90]. It has been proposed by Ruiz et al. that the IVIG effect on inhibition of NK cells cytotoxicity in vitro is mediated only by the effector cell not by the target cell; and this process may

Fig. 2 Classic complement activation and action of IVIG

involve antibody dependent cellular cytotoxicity and may also involve alteration of cytokine production [91]. Furthermore, an *in vivo* study showed that decline in number of elevated blood CD3⁺ CD56⁺ NKT cells in response to IVIG treatment correlates with successful pregnancy. This study brought to two interesting results at once: the importance of a specific subpopulation of NK cells, NKT cells, in pregnancy failure, both in pathogenesis and treatment; and identification of patients that more likely may benefit of IVIG [92].

Finally, in women with repeated IVF failure and the positivity for APE and APS, empiric treatment with IVIG was reported to be beneficial. In this condition, as it was already reported that APE and APS presence is related to NK cell activity, it is controversial whether IVIG act on the APL or most likely, by reducing NK cells number and activity [73].

It has been proposed a complex panel to identify patients with RSA responders to IVIG therapy, comprising human leukocyte antigen typing, FcR-blocking antibody and TLX type, cell-mediated lympholysis, partner-specific cell-mediated lympholysis activity, partner-specific cytotoxic T lymphocyte precursor frequency, NK cell-51Cr release assay, and NK cell activity, enzyme-linked immunosorbent assays for anti-dsDNA autoantibody and antiphospholipid antibodies. This study revealed that RSA patient show high total capacity of iron bounding in peripheral blood and a HLA class I mismatch, a high cytotoxic potential that may be associated with the lack of erythrocytes antibody inhibition-blocking antibody, that appeared after IVIG therapy and were associated with the continuation of the pregnancies [93].

NK cells playmakers in pregnancy

It is evident that whether NK cells are protective rather than pathogenic remains controversial, however, functional differences between NK cells at different anatomical local-

izations and the adaptability, or flexibility, of effector responses in NK cells may also explain differences in such responses. This is evident considering the previous observations: PNK and UNK are dramatically different populations with different tasks, and peripheral blood or migration in the deciduas is diriment in addressing NK cell type. Cytokine milieu and other stimuli acting on different cell surface receptors such as KIR, present in the target organ, may differently trigger NK cells response and influence their role. And also, the microenvironment in which they mature triggers differential effector responses in NK cells as different molecules have unique effects on NK cell effector functions, as clearly demonstrated by diverse effects exerted by IL-5, IL-6, IL-15, TNF- α , human chorionic gonadotrophin (β HCG), progesterone, and prolactin during pregnancy.

Conclusions

In short, NK cell alteration may be associated with impaired pregnancy, and the modulation in the number of circulating NK cells is most likely to be a primary event rather than an active inflammation/drug administration consequence during an inflammatory/autoimmune process, thus playing an important role in the pathogenesis of immunological infertility. NK cell functions at different anatomical sites as well as the role of genetic factors, especially in terms of response to different stimuli coming from the local microenvironment to which NK cells home and become activated should be deeper investigated.

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