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The Clinical Significance of Eosinophils in the Amniotic Fluid in Preterm Labor

ROBERTO ROMERO[1,2,3], **JUAN PEDRO KUSANOVIC**[1,2], **RICARDO GOMEZ**[4], **RONALD LAMONT**[1,2], **EGLE BYTAUTIENE**[5], **ROBERT E. GARFIELD**[6], **POOJA MITTAL**[1,2], **SONIA S. HASSAN**[1,2], and **LAMI YEO**[1,2]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, Maryland, and Detroit, Michigan, USA

[2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan, USA

[3]Center for Molecular Medicine and Genetics, Wayne State University, Detroit, Michigan, USA

[4]Department of Obstetrics and Gynecology, CEDIP (Center for Perinatal Diagnosis and Research), Sotero del Rio Hospital, P. Universidad Catolica de Chile, Santiago, Chile

[5]Department of Obstetrics and Gynecology, The University of Texas Medical Branch, Galveston, Texas, USA

[6]Department of Obstetrics and Gynecology, St. Joseph's Hospital and Medical Center, Phoenix, Arizona, USA

Abstract

Objective—White blood cells are not traditionally considered to be normally present in amniotic fluid. This study was conducted after the observation that a patient with preterm labor and intact membranes had eosinophils as a predominant cell in the amniotic fluid, and had an episode of asthma during the index pregnancy. The goal of this study was to determine whether women presenting with preterm labor with eosinophils in the amniotic fluid had a different outcome than those without eosinophils as the predominant white blood cell in the amniotic cavity.

Methods—This retrospective case-control study included women who presented with preterm labor and intact membranes between 24 and 34 weeks of gestation. Patients underwent an amniocentesis shortly after admission for the assessment of the microbiologic status of the amniotic cavity and/or fetal lung maturity. Amniotic fluid was cultured for aerobic and anaerobic bacteria as well as genital mycoplasmas. Cytologic studies included amniotic fluid white blood cell count and differential, which was performed on cytocentrifuged specimens. Patients with microbial invasion of the amniotic cavity and/or a white blood cell count >20 cells/mm^3 were excluded from the study. Cases were defined as women in whom the differential contained >20% of eosinophils. Controls were selected among women with an amniotic fluid eosinophil count ≤20% and matched for gestational age at amniocentesis. The analysis was conducted with non-parametric statistics.

Results—The study population consisted of 10 cases and 50 controls. Gestational age and cervical dilatation at admission were similar in both groups. Cases had a lower gestational age at delivery than controls [34.6 weeks, inter-quartile range (IQR) 32–37.3 weeks vs. 38.0 weeks, IQR 35–40 weeks, respectively; p=0.018]. The prevalence of preterm delivery ≤35 weeks was higher

Address correspondence to: Roberto Romero, M.D. Perinatology Research Branch, NICHD, NIH, DHHS Wayne State University/ Hutzel Women's Hospital 3990 John R, Box 4 Detroit, MI 48201, USA Telephone (313) 993-2700 Fax: (313) 993-2694 prbchiefstaff@med.wayne.edu.

among patients who had >20% eosinophils than in the control group [50% (5/10) vs. 18% (9/50), respectively; p=0.029]. Similar results were observed for delivery at <37 weeks [Cases: 70% (7/10) vs. Controls: 36% (18/50); p=0.046].

Conclusions—Women with preterm labor and intact membranes who have a large proportion of eosinophils in the amniotic fluid are at an increased risk for spontaneous preterm delivery. These patients may have had an episode of preterm labor related to a type I hypersensitivity reaction.

Keywords

Premature birth; preterm birth; prematurity; premature labor; mast cells; amniotic fluid cells; amniotic fluid white blood cells; allergy; allergy-induced preterm labor; atopy; pregnancy; type I hypersensitivity reaction; parturition; labor; eosinophil granule proteins

INTRODUCTION

Preterm parturition is a syndrome[1] caused by multiple etiologies, including intrauterine infection/inflammation,[2–24] uteroplacental ischemia,[25,26] cervical disorders,[27–29] uterine overdistension,[30] abnormal allograft reaction,[31] endocrine disorders,[32–35] and other causes. Some forms of preterm labor remain idiopathic in nature. In other words, no clear mechanism of disease can be implicated.

More than 20 years ago, one of the authors (RR) performed an amniocentesis in a patient presenting with preterm labor and intact membranes, and the laboratory reported that the predominant cell in the fluid were eosinophils. The laboratory staff believed that this was a mistake, and suspected that the fluid was erroneously labeled in the laboratory as amniotic fluid rather than tracheo-bronchial lavage, and requested that additional fluid be sent for reexamination. After explaining to the patient what had transpired, a second amniocentesis was performed and the laboratory confirmed that, in fact, eosinophils were the predominant cells in the amniotic fluid (Figure 1). Upon further conversation with the patient she expressed that developed asthma for the first time during this pregnancy. After this observation and consideration of the potential implications, a retrospective study was performed to determine the clinical significance of eosinophils in the amniotic cavity. This study was presented at the Society of Perinatal Obstetricians in 1991.[36] Since that time, the authors have received numerous calls from the United States and abroad, in which amniocenteses of patients in preterm labor have revealed eosinophils. We decided to share these results with the scientific community because there is no literature to provide guidance to physicians, and we have been asked to advice as to the clinical significance of this finding. This study was conducted to address this question.

MATERIALS AND METHODS

Study population

A retrospective case-control study was conducted by searching our perinatal database to identify women who were admitted with the diagnosis of preterm labor with intact membranes and met the following criteria: 1) singleton gestation; 2) gestational age between 24 and 34 completed weeks; and 3) amniocentesis with a negative culture, a white blood cell count of <20 cells/mm^3 and a differential available. Spontaneous preterm labor was defined by the presence of regular uterine contractions occurring at a frequency of at least two every 10 minutes associated with cervical change before 37 completed weeks of gestation that required hospitalization. Women were cared for at Hutzel Hospital, Detroit, Michigan. Cases were women in which the differential showed that eosinophils represented >20% of the white blood cells in the amniotic fluid. Controls were patients in which the amniotic fluid

differential contained ≤20% of eosinophils. Patients were matched for gestational age at the time of amniocentesis. All women provided written informed consent prior to the collection of amniotic fluid. The collection and utilization of amniotic fluid for research purposes was approved by the Institutional Review Boards of the participant institutions and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS.

Amniotic fluid studies

Amniotic fluid was obtained by transabdominal amniocentesis under ultrasonographic guidance. The fluid was transported to the laboratory in a capped plastic syringe and cultured for aerobic and anaerobic bacteria, as well as for mycoplasmas. White blood cell count, glucose concentration and Gram-stain were also performed shortly after collection. Cytologic studies included cell count and differential, which was performed by trained, technical personnel in the laboratory at the Detroit Medical Center. Cytocentrifuged smears of amniotic fluid stained with Wright-Giemsa were used for this purpose. Amniotic fluid not required for clinical assessment was centrifuged for 10 minutes at 4°C and the supernatant was aliquoted and stored at - 70°C until analysis.

Statistical analysis

The normality of the data was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Comparisons between proportions were performed with the Chi-square test, and Mann-Whitney U tests were used for continuous variables. A Kaplan-Meier survival analysis was conducted to assess the amniocentesis-to-delivery interval. Spontaneous labor was entered in the analysis as the event of interest, and patients who delivered due to fetal or maternal indications were treated as censored observations with a censoring time equal to the amniocentesis-to-delivery interval.

RESULTS

The study population consisted of 10 cases and 50 controls. Table I displays the clinical characteristics of the study population. No differences were observed in the median gestational age at amniocentesis (30.4 weeks vs. 30 weeks, respectively; p=0.7) and median cervical dilatation at admission (1.5 cm vs. 1.0 cm, respectively; p=0.5) between cases and controls.

Patients with more than 20% eosinophils in the amniotic fluid white blood cells differential count had a lower median gestational age at delivery than controls [Cases: 34.6 weeks, inter-quartile range (IQR) 32–37.3 weeks vs. Controls: 38.0 weeks, IQR 35–40 weeks; p0.018]. The frequency of preterm birth before 35 weeks of gestation was higher among cases than in controls [Cases: 50% (5/10) vs. Controls: 18% (9/50); p=0.029]. Similar results were observed for delivery at less than 37 weeks of gestation [Cases: 70% (7/10) vs. Controls: 36% (18/50); p=0.046].

Although the amniocentesis-to-delivery interval was not significantly different, the median difference between cases and controls was 7 days [Cases: 34 days (95% CI 0–70 days) vs. Controls: 41 days (95% CI 34–48 days); p=0.084] (Figure 2).

COMMENTS

Principal Findings of the Study

1) A subset of patients with preterm labor and intact membranes have eosinophils in the amniotic fluid; 2) patients with an amniotic fluid white blood cell count differential containing more than 20% of eosinophils are at an increased risk of preterm delivery; 3)

some patients with eosinophilis in amniotic fluid delivered at term and had no evidence of complications.

White Blood Cells in the Amniotic Fluid

Under normal circumstances, clinical laboratories do not report white blood cells in the amniotic fluid obtained in the midtrimester or in patients undergoing amniocentesis at term for fetal lung maturity studies without contamination with maternal blood. The first study[37] in which we examined the clinical value of an amniotic white blood cell count and differential as a rapid method for the diagnosis of microbial invasion of the amniotic cavity, and also for the detection of intra-amniotic inflammation, indicated that the most frequent cell was granulocytes, and specifically, neutrophils. We observed that some patients had lymphocytes, and others had macrophages. Yet, eosinophils were not described in that report (195 patients), which was conducted over a 31-month period at Yale-New Haven Hospital/ Yale University.[37]

The potential importance of eosinophils in the amniotic fluid came to our attention with the case described in the introduction of this manuscript, and which justified the current study. The patient admitted with preterm labor and intact membranes and had eosinophils in the amniotic fluid as a predominant cell, presented after that study[37] was completed. After that time, we conducted a study to systematically examine the clinical significance of eosinophils in the amniotic fluid of patients with preterm labor and intact membranes. This study was presented at the Society for Perinatal Obstetricians in 1991,[36] and we observed that women with eosinophils were more likely to have a history of atopy (e.g. allergic rhinitis, atopic dermatitis, and 2 with asthma for the first time in the index pregnancy). The data from that study was misplaced by staff working at another institution located at Washington, D.C. Since eosinophils are elevated in peripheral blood and other biological fluids in the context of allergic reactions, it was proposed that eosinophils in the amniotic fluid may represent evidence of an “allergic-like” response in the uterus.[36] A few patients that we studied at Yale-New Haven Hospital and who were found to have eosinophils in the amniotic fluid had their stool examined for ova and parasites, and the results were negative. Therefore, we have no evidence of helminthic infections in these patients.

Eosinophils

These cells are bone-marrow derived, multi-functional leukocytes, actively motile, and terminally differentiated (e.g. non-dividing cells).[38] The nucleus of the cell is bi-lobed, and the cytoplasm contains specific granules which have arginine-rich basic proteins, which are colored bright orange by acidic dyes such as eosin.[39] This is what gives eosinophils their name and typical appearance under the microscope. The staining properties of eosinophils were discovered by Paul Ehrlich and presented to the Berlin Physiologic Society in 1878.[40] The cytoplasm of eosinophils contains primary granules, secondary granules, lipid bodies, and Charcot-Leyden crystals, which are only seen during activation.[41] The primary granules are the main site of Charcot-Leyden crystal protein, which is also called galectin-10. This glycoprotein accounts for 10% of the total cellular proteins in the mature blood eosinophil. The secondary granule contains major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil-derived neurotoxin (EDN), as well as several cytokines.[42]

Eosinopoiesis occurs mainly in the bone marrow, and other sites of production include the spleen, the thymus, and the lymph nodes.[43] The post-mitotic eosinophil reservoir capacity is approximately $9–14 \times 10^8$ cells per kilogram.[44] The development of multipotential cells into eosinophil progenitors is thought to result from a stochastic process,[43] followed by survival which is supported by several cytokines including interleukin (IL)-3, IL-4, IL-5,

stem cell factor, granulocyte-macrophage colony stimulating factor (GM-CSF), and eotaxin. These cytokines sustain the terminal stages of eosinophil maturation and release into the bloodstream.[45,46] The bone marrow of healthy individuals contains about 3% of eosinophils, of which 37% are mature, up to 16% of eosinophilic myelocytes are in the S-phase of the cell cycle (which lasts approximately 13 hours). The time elapsed between the last mitosis and the appearance of eosinophils in blood as mature cells is approximately 2.5 to 3.5 days.[42,44,47] The average residence of an eosinophil in the bone marrow is 3–5 days. The half-life of eosinophils in the circulation is about 18 hours, and the mean blood transit time is approximately 26 hours.[48]

Most of the total eosinophil pool (99%) is contained in connective tissues, and eosinophils represent less than 4% of circulating white blood cells.[41] Circulating eosinophils migrate into tissues to exert their biological properties and participate in inflammatory processes elicited by parasitic helminth infections, and allergic diseases.[49,50] Eosinophils are normal constituents of the mucosal immune system of the gastrointestinal, respiratory, and urogenital tracts.[49]

Eosinophils are derived from a pluripotent stem cell, which differentiates into a common precursor of basophils and eosinophils. Then, there is further differentiation into a separate eosinophil lineage.[51] Three cytokines play a role in regulating eosinophilic development (sometimes, they are collectively referred to as “eosinopoietins”): IL-3, IL-5, and GM-CSF.[52–55] IL-5 is the most specific to the eosinophil lineage, can stimulate the release of eosinophils from the bone marrow into the peripheral circulation,[56]and transgenic mice for IL-5 results in substantial eosinophilia.[57–60] In addition to the role of IL-5, the most important chemokine that regulates the traffic of eosinophils into tissues under physiologic conditions is eotaxin-1.[49] This chemokine and its receptor are important under physiologic conditions for the delivery of eosinophils to the mucosa of the gastrointestinal tract, mammary gland, thymus, and uterus (endometrium).[50,61–67]

The role of eosinophils in the endometrium is believed to be related to estrus cycling.[49,68] Indeed, eotaxin-1 peripheral concentrations change in response to estrogens, and the number of eosinophils in the endometrium changes during the menstrual cycle.[38,50] Moreover, gene deletion of IL-5 decreases the number of eosinophils in the blood,[69,70] as well as in the endometrium (4 to 7-fold) in the estrus cycle and in early pregnancy.[66] Similarly, eosinophils in the cervix and decidua are decreased in IL-5 knockout mice. Yet, eosinophils have been found in the endometrium of these animals, suggesting that a subpopulation of eosinophils is independent of IL-5. Interestingly, the onset of parturition is not affected in IL-5 knockout mice.[66]

Under pathologic conditions, several chemokines and cytokines are involved in the migration of eosinophils to inflammatory sites.[38,49,50,71] Eosinophils exert part of their biological properties through the release of proteins from intracellular granules. These proteins, called eosinophil-granule proteins, include: 1) major basic protein; 2) eosinophilic cationic protein; 3) eosinophilic peroxidase; and 4) eosinophilic-derived neurotoxin. These proteins are stored in the specific granules, and they are toxic to parasite larvae and to some mammalian cells. ECP and EDN are ribonucleases, and have anti-viral activity. ECP can induce mast cell degranulation, and suppress T-cell proliferative responses. MBP also induces mast cell and basophil degranulation. EPO constitutes 25% of the total protein mass of specific granules, and is involved in the formation of reactive oxygen species and reactive nitrogen metabolites. Consequently, they can be involved in promoting oxidative stress and cell death.[49] Eosinophilic proteins are considered to be almost exclusive to these cells. Plasma concentrations of MBP are 10 to 20 times higher in pregnant than in non-pregnant women.[72] Moreover, a relationship was reported between MBP concentrations and the

impending onset of labor at term (in one case in preterm labor).[72] Since eosinophil numbers do not change during pregnancy, an alternative source of these proteins was searched and found in the placenta.[73,74] A role for MBP in labor was postulated because this protein has been localized to interstitial trophoblasts, which are in close proximity to the myometrium. It has been postulated that these cells may play a role in the onset of labor because of their proximity to the myometrium.[75]

Recently, a condition known as “eosinophilic/T-cell chorionic vasculitis” was described.[76] This condition is characterized by focal infiltration of chorionic vessels (artery or vein) by eosinophils, but its clinical significance remains to be determined. Redline reported that recurrent villitis associated with bacterial bacilli (demonstrated by Steiner stain) included macrophages, lymphocytes, plasma cells and neutrophils, as well as eosinophils.[77] Indeed, eosinophils can be observed in cases of acute chorioamnionitis. Eosinophils have been demonstrated in the placenta of animals having adverse pregnancy outcome. One case included preterm birth in an alpaca (*Lama pacos*) which delivered at 290 days of gestation (normal gestation 335–360 days) and had placentitis with a cellular infiltration by lymphocytes, eosinophils and neutrophils in the chorionic membranes.[78] Gram negative, period acid-Schiff positive, variably acid-fast spores were observed. Using molecular microbiologic techniques, encephalitozoon cuniculi-associated placentitis was identified using PCR and sequencing of the PCR products.[78] A second case of an infection in an alpaca was reported shortly after the animal had a spontaneous abortion.[79] In this case, the mother died after the abortion, and the autopsy showed disseminated eosinophilic myositis. Large cysts were observed and attributed to an infection with *Sarcocystis sp*. (probably *Sarcocystis aucheniae*); however, this organism was not cultured. There was no evidence of infection in the placenta or uterus.[79]

Eosinophils in the Amniotic Fluid

It is not known whether eosinophils in the amniotic fluid are of fetal or maternal origin. Similarly, the chemotactic stimulus that brings eosinophils into the amniotic fluid has not been identified and their role in the amniotic cavity is not known. Eosinophils have an important role against viruses[80–84] and it is possible that these cells participate in host-defense against viral infections. However, the study of the viral burden and diversity in amniotic fluid is in its infancy. We would like to emphasize that the purpose of this report is simply to call attention to the unexpected observation of finding eosinophils in the amniotic fluid. We have performed this study using the results reported by the clinical laboratory of the Detroit Medical Center during the course of clinical care. Therefore, eosinophils were identified in a routine cytocentrifuged smear. We have not conducted an eosinophil count, which has been used in the study of eosinophil biology and the diagnosis of hypereosinophilic syndromes.[85] Similarly, we are cautious in interpreting the biological meaning of our results.

Could Amniotic Fluid Eosinophils be a Marker for an Inflammatory Reaction and if So, What Type of Inflammatory Reaction?

Eosinophils are involved in the late phase of a Type I Hypersensitivity reaction (or allergic reaction).[86] Eosinophils are capable of creating an environment that stimulates mast cell degranulation (eosinophils incubated with the mast cell protease “chymase” can induce the production of “eosinophil-derived stem cell factor”, which is capable of promoting the growth of mast cells). Moreover, they are considered to be responsible for tissue damage that occurs during Type I Hypersensitivity reaction. Eosinophils can secrete collagenases, proteases, and peroxidases, and they may participate in tissue damage within the amniotic cavity. The arguments and evidence that a Type I Hypersensitivity reaction may participate as a cause of preterm labor have been described elsewhere (*Romero R. et al., submitted*).

Whether or not patients with eosinophils in amniotic fluid are the same as those having an episode of preterm labor induced by an allergen remain to be determined.

Some Mysteries About Eosinophils

The traditional view is that the toxic products of eosinophils contribute to the killing of helminths, a view that can be traced back to the observation that the schistosomula of *Schistoma mansoni* can undergo eosinophilic–dependant killing.[87] Although eosinophils have been traditionally considered to provide protection to the host during parasitic infections, a recent study has called for a reexamination of this idea. This year, Fabre et al. [88] published a study in which eosinophils were required for the survival of the parasite. The experiments were conducted with *Trichinella spiralis*, a worm that is naturally found in mice, and produces a chronic infection in skeletal muscle. The parasite was introduced into wild-type mice as well as two strains lacking eosinophils. These cells were found to infiltrate infected muscles in wild-type mice. In contrast, *Trichinella spiralis* larvae die in large numbers in mice without eosinophils. The authors suggest that eosinophils may be important in establishing a chronic infection that would allow survival of the worm in the host.[88] Whether this is the case in humans as well as for other helminths remains to be determined.

The other major role assigned to eosinophils is to participate in allergic reactions.[38] In this inflammatory process, eosinophils have been considered to play a secondary role to helper type 2 (Th2) immune responses.[89] Yet, recent studies conducted with mice devoid of eosinophils indicate that Th2 cytokines are lower in these animals than in controls when challenged with an allergen.[90] These observations suggest that in the lung, eosinophils may be required for localized recruitment of effector T cells. If this is correct, then the role of eosinophils in the initiation of a Type I Hypersensitivity reaction would also need to be re-examined.

The phylogeny of eosinophils is also interesting. All vertebrates appear to have them. Eosinophils have been found in fish, amphibians, birds, and reptiles. In many of these classes, the eosinophil granules appear somewhat different in that they do not contain a crystalloid internum.[91] The precise role of this cell in these species remains to be determined.

Several investigators have reported the presence of eosinophilic proteins, such as ECP, in human amniotic fluid.[92–94] The sources and physiologic role of these proteins remains to be determined. Recently, the proform of eosinophil major basic protein (ProMBP) has been demonstrated to exist in the serum from pregnant women (and also in non-pregnant women) complexed with a variable fraction of angiotensinogen. Moreover, a subfraction binds to complement C3dg.[95] Yet, the clinical significance of ProMBP is unknown.

Recently, eosinophils have been proposed to play a role in innate immunity by recognizing the presence of “danger signals”.[96] The danger theory of immunity was proposed by Matzinger,[97] and postulates that the immune system recognizes molecules and cells capable of causing danger to the host rather than to discriminate between self and non-self. Therefore, these molecules can arise not only from microorganisms (non-self), but also from the host owned cells, which are damaged or stressed. The typical case would be a cell in the process of necrosis. Recent observations suggest that eosinophils from healthy individuals can recognize danger signals (necrotic intestinal cells) because such material was able to induce chemotaxis of eosinophils and the release of eosinophil peroxidase.[96] This proposal is interesting because it assigns to the eosinophil a constructive role in immune defense, rather than a destructive role, which is the reputation that eosinophils have enjoyed for several decades. The precise role of these cells may be tissue repair, given that they can

produce growth factors such as fibroblast growth factor, nerve growth factor, vascular endothelial growth factor, and TGF-β1.[98–101] Since eosinophils express Toll-like receptor 4 as well as CD14, they can respond to bacterial endotoxin.[102,103] It is noteworthy that in the late 1800s, Paul Ehrlich had suggested that eosinophils could be activated by toxic products of foreign organisms or products of tissue breakdown.[104]

Eosinophilic Granulocytes and Damage-Associated Molecular Associated Patent Proteins (DAMPs)

Recent evidence suggests that eosinophils can act as antigen-presenting cells. Lucey et al. [105] demonstrated that eosinophils can express HLA-DR, and that this subset of cells can induce lymphocyte proliferation.[106] Moreover, adoptive transfer of antigen-pulsed eosinophils can induce antigen-specific T-cell responses *in vivo*.[106,107] An important discovery is that incubation of eosinophils with the high mobility group box 1 protein (HMGB1) induces up-regulation of HLA-DR and CD86. HMGB1 is a highly conserved protein present in the nuclei and cytoplasm of nearly all cell types, which is considered upon release of necrotic, but not apoptotic death of normal cells. HMGB1 is released by several immune and non-immune cells. HMGB1 is considered a “danger signal” for eosinophils. The administration of this protein to normal animals causes a systemic inflammatory response, including fever, acute lung injury, epithelial barrier dysfunction and death. Anti-HMGB1 treatment can rescue mice from lethal endotoxemia, or sepsis.[108] HMGB1 is a constituent of chromatin proteins and has chemotactic properties. Thus, Lotfi et al.[42] have argued that HMGB1 is ideally positioned to be a unique “danger signal” which identifies cells of regions of cellular stress/necrosis and attract granulocytes, including neutrophils. Indeed, HMGB1 can attract eosinophils, serve as a survival factor by itself, or in combination with eotaxin.[42] We believe that the expression of DAMPs within the amniotic cavity or in the uterine cavity may explain the influx of eosinophils in patients without infection. DAMPs may be induced by non-microbial related agents, under conditions of hypoxia or oxidative stress. This proposal provides a link between the presence of eosinophils in the amniotic fluid and a wide range of insults within the amniotic cavity.

Finally, eosinophils can also serve an immunoregulatory role. These cells can produce a number of cytokines, such as interferon gamma (Th-1), or members of the Th-2 family, such as IL-4, IL-5, IL-13. In addition, it can produce members of the Th-3 family, such as TGF-β and IL-10, and possibly, members of the Th-4 family of cytokines which includes IL-17, also known as Th-17.[109–111] Another role for eosinophils is to modulate local T-cell activity. This can be accomplished by the production of eosinophil-derived indoleamiane 2,3-dioxygenase (IDO) production of kynurenine.[112]

The Biological Complexity of Eosinophils

The traditional view that eosinophils were predominantly involved in helminth and parasite diseases has given way to a different view in which these cells play a more complex role in tissue hemostasis. Eosinophils have been implicated in the pathogenesis of a very long list of disease states, including autoimmune diseases, and even cancer. Lotfi et al.[42] has proposed that the current confusion about the role of eosinophils derived from the fact that these cells have evolved as part of host inflammatory/remodeling mechanism, and therefore, the specific role of these cells depends upon circumstances and that their primary function is to maintain tissue hemostasis.

Conclusion

Eosinophils can be found as the predominant cell in the amniotic fluid of a small subset of women with preterm labor and intact membranes. These patients are at increased risk for

preterm delivery. Yet, many experience an episode of preterm labor and are discharged and deliver at term. Further studies are required to determine the role of eosinophils in the amniotic fluid, as well as the physiology of proteins which have been traditionally considered as eosinophilic-specific.

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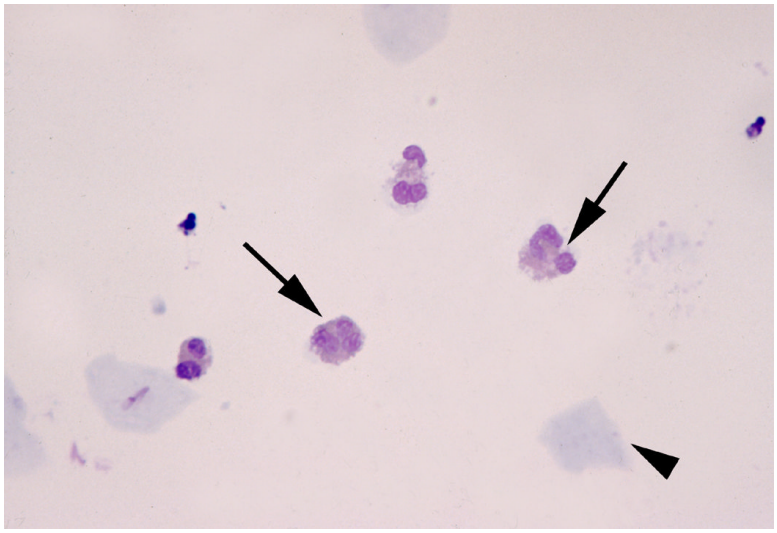

Figure 1. Eosinophils in the amniotic fluid

Amniotic fluid obtained from a patient with spontaneous preterm labor and intact membranes. The eosinophils (*arrows*) were the predominant cells in the amniotic fluid (*arrowhead*: epithelial cell).

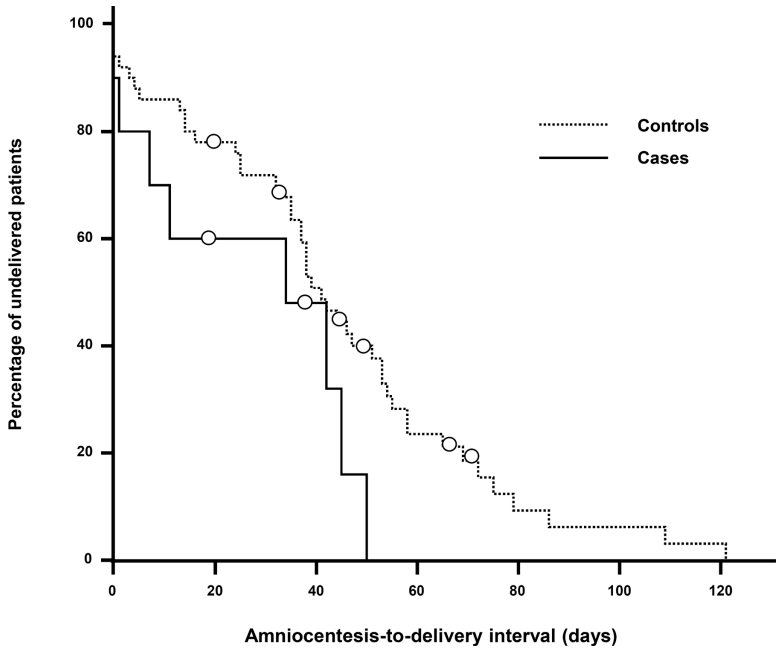

Figure 2. Amniocentesis-to-delivery interval according to the presence or absence of amniotic fluid white blood cell count differential of more than 20% of eosinophils

Although the difference was not significant, the median amniocentesis-to-delivery interval was seven days shorter in cases than controls [Cases: 34 days (95% CI 0–70 days) vs. Controls: for cases and 41 days (95% CI 34–48 days); p=0.084]. *Solid line*: cases; *dashed line*: controls; *open circles*: censored patients.

Table I

Clinical characteristics of the study population

	Cases (n=10)	Controls (n=50)	p
Nullipara	20 (2/10)	32 (16/50)	0.5
Previous preterm delivery	10 (1/10)	20 (10/50)	0.5
Gestational age at amniocentesis (wks)	30.4 (27–34.1)	30 (28.6–31.3)	0.7
Cervical dilatation at admission (cm)	1.5 (1–3)	1.0 (1–3)	0.6
AF WBC count (cells/mm^3)	6 (5–8)	2 (0–9)	0.019
AF glucose (mg/dL)	28 (25–43)	36 (28–42)	0.3
Gestational age at delivery (wks)	34.6 (32–37.3)	38.0 (35–40)	0.018
Preterm delivery ≤ 35 weeks	50 (5/10)	18 (9/50)	0.029
Preterm delivery ≤ 37 weeks	70 (7/10)	36 (18/50)	0.046
Birthweight (grs)	2,048 (1,860–3,033)	2,693 (2,011–3,125)	0.1

Results are expressed as median (inter-quartile range) and percentage (proportion) AF: amniotic fluid; WBC: white blood cells