Diplomarbeit

The role of immune cells in the pathogenesis of endometriosis: a possible target for therapy?

eingereicht von

Isabella Rosenauer

zur Erlangung des akademischen Grades

Doktorin der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Otto-Loewi-Forschungszentrum, Lehrstuhl für Pharmakologie

unter der Anleitung von

Priv.-Doz. Mag. Dr.rer.nat Luschnig Petra

Univ.-Prof. Dr.med.univ. Heinemann Akos

Graz, 24.10.2022

Eidesstaatliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 24.10.2022 *Isabella Rosenauer eh*

Danksagung

An dieser Stelle möchte ich mich bei all denjenigen bedanken, die mich während der Ausarbeitung dieser Diplomarbeit unterstützt haben.

Ein großer Dank gebührt meiner Betreuerin Frau Prof. Luschnig. Für die hilfreichen und konstruktiven Anregungen bei der Erstellung der Arbeit. Sie hat nicht nur meine Arbeit betreut, sondern mich auch unterstützt und motiviert. Dafür möchte ich mich herzlich bedanken.

Außerdem möchte ich mich bei dem Otto-Loewi-Forschungszentrum, für die Möglichkeit meine Diplomarbeit so umzusetzen, bedanken.

Ebenfalls möchte ich mich bei meinem Mann für den starken emotionalen Rückhalt über die Dauer meines gesamten Studiums bedanken.

Abschließend möchte ich mich bei meinen Eltern bedanken, die mir mein Studium durch ihre Unterstützung ermöglicht haben und stets ein offenes Ohr für mich hatten.

Inhaltsverzeichnis

List of abbreviations

Abbreviation	Meaning
1-MT	1-methyl-tryptophan
B-cells	B-lymphocytes
BLyS	B-lymphocyte stimulator
BTG-1	B-cell translocation gene 1
Btk	Bruton-tyrosine-kinase
CA125	Tumour marker for ovarian cancer
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCL10	Interferon-γ-induced protein 10
DAMP	Damage associated molecular patterns
DC	Dendritic cells
FSH	Follicle-stimulating hormone
G-CSF	Granulocyte-colony-stimulating-factor
GnRH	Gonadotropin-releasing hormone
HLA	Human leukocyte antigens
IDO1	Indolamine 2,3-dioxygenase
IFN-γ	Interferon γ
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin 1
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-12B	Interleukin 12B
IL-15	Interleukin 15
IL-17A	Interleukin 17A
IL-1β	Interleukin 1β
IL-2	Interleukin 2
IL-22	Interleukin 22
IL-23	Interleukin 23
IL-3	Interleukin 3
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-8	Interleukin 8
ILC	Innate lymphoid cells
KIR	Killer cell immunoglobulin-like receptor
LH	Luteinising hormone
LT	Leukotrienes
MHC-I	Major histocompatibility complex I
MHC-II	Major histocompatibility complex II
MR	Magnetic resonance
MUC1	Mucin 1 glycoprotein
NET	Neutrophil extracellular traps
NK-cells	Natural killer cells
NKT	Natural killer-like T-cells
NLR	Neutrophil-to-lymphocyte ratio

NOS	Nitric oxide synthase
PAF	Platelet activating factor
PAMP	Pathogen associated molecular patterns
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death protein-1 ligand
PG	Prostaglandin
PRR	Pattern recognition receptors
RANTES	Regulated on activator normal T-cell expressed and secreted
ROS	Reactive oxygen species
T-cells	T-lymphocytes
TECK	Thymus-expressed chemokine
TGF-β	Transforming growth factor β
Tie2^{+}	Tunica intima endothelial kinase 2 (special type of macrophages)
TNF-α	Tumour necrosis factor α
Treg	Regulatory T-cells
uNK	Uterine natural killer cells
VCAM-1	Vascular adhesion molecule-1
VEGF	Vascular endothelial growth factor

Abstract

Endometriosis is a disease affecting many women worldwide. Despite the high incidence of endometriosis, little is known of its origin. Especially the etiology of endometriosis is not known yet, although many theories exist. Since changes of the immune cells in endometriosis have been observed, their role in the emergence and formation of the disease has been under discussion. In many studies, different types of immune cells have been proven to be increased or decreased in patients with endometriosis.

The aim of this thesis was to find out if immune cells have an impact on the emergence of endometriosis. For this reason, the results of different publications were summarised and compared to determine, if there is a certain connection. This connection could be a target for medical therapy in the future, which highlights the importance of the analysis. In the center of attention are macrophages, lymphocytes, especially T-cells and natural killer cells. PubMed has been used as the main source of scientific research literature. The found studies have been divided by the different cell types and compared. Especially, studies showing differences in the quantity of immune cell types in endometriosis, regarding their function, indicates an important role of the immune system in endometriosis. In addition to that, a few studies including mouse models detected changes in endometriosis tissue after blocking different immune cell types or receptors interacting with immune cells. These studies indicate a promising treatment approach for the immune system in endometriosis.

Zusammenfassung

Endometriose ist eine weitverbreitete Krankheit unter Frauen über die noch sehr wenig bekannt ist. Vor allem die Ätiologie der Endometriose beschäftigt die Wissenschaft und es wurden verschiedene Theorien dahingehend formuliert. Die Ätiologie zu verstehen ist wichtig, um mögliche Therapieansätze zu finden und somit für viele Frauen eine mögliche Therapie anbieten zu können. In dieser Arbeit wird auf eine mögliche Entstehungstheorie, die das Immunsystem als ursächlich formuliert eingegangen. Diese Theorie war in den vergangenen Jahren von großem wissenschaftlichem Interesse. Die große Anzahl der Forschungsergebnisse zum Immunsystem im Zusammenhang mit Endometriose ist unübersichtlich, deshalb war das Ziel dieser Arbeit eine Zusammenfassung dieser Studienergebnisse zu liefern und somit einen besseren Überblick zu erhalten. PubMed wurde als Hauptquelle für wissenschaftliche Veröffentlichungen verwendet und es wurden die neuesten und relevantesten Studien verglichen. Um eine gute Übersicht zu erhalten, wurde die Arbeit in die einzelnen Immunzellen eingeteilt und diese in den jeweiligen Kapiteln genauer betrachtet.

Vor allem die angenommenen Aspekte, die zur Entstehung von Endometriose beitragen könnten, wie Angiogenese, cytotoxische Aktivität und Ähnlichkeiten zu Autoimmunerkrankungen wurden genauer betrachtet. Die Ergebnisse aus verschiedenen Studien zu verminderten oder erhöhten Immunzellen in der Peritonealflüssigkeit oder auch den Endometriose Läsionen in Zusammenschau mit der grundsätzlichen Funktion der Zellen lassen darauf schließen, dass das Immunsystem eine wichtige Rolle für Endometriose spielt. Besonders hervorzuheben sind einige Studien, die beispielsweise in Mausmodellen bestimmte Zellen oder Rezeptoren, die im Zusammenhang mit dem Immunsystem stehen, blockierten. Diese zeigten Veränderungen in der Läsionsentstehung, Größe oder auch im Gewicht der Läsionen, weshalb man eine Rolle des Immunsystems in der Endometriose Entwicklung annehmen kann. Jedoch ist es schwer zu sagen, ob der Einfluss des Immunsystems bei der Entstehung oder Entwicklung der Erkrankung von Wichtigkeit ist. Diese Studien zeigen allerdings, dass das Immunsystem als Therapieansatz erfolgversprechend sein könnte.

1. Introduction

1.1 Endometriosis

Endometriosis is a gynaecological disease in reproductive women. It is characterized by the occurrence of ectopic endometrium or endometrial tissue, which means that endometrial glands and stroma, a physiological tissue layer of the uterus, are situated outside of the uterus. Etiology and pathophysiology are not scientifically proven yet. So far, a few etiologic theories exist, but none are clarified until now. The complexity of this disorder and the different locations where the endometrial lesions occur represent a massive challenge for research (1).

Depending on the location of ectopic endometrial tissue, three different types of endometriosis are distinguished: i) endometriosis genitalis externa, ii) endometriosis genitalis interna and iii) endometriosis extragenitalis (1).

i) Endometriosis genitalis externa occurs within the organs of the small pelvic. Especially the ovaries are affected as well as the ligaments of the uterus and the tissue within the douglas room. The following text will mainly deal with this type of endometriosis since it occurs most frequently and is therefore of big scientific interest.
ii) In endometriosis genitalis interna the endometrial lesions can be found in myometrial tissue. This type of endometriosis is also called Adenomyosis uteri.
iii) The ectopic tissue in the third category, Endometriosis extragenitalis, is situated outside of the genital organs. This one is the least common type and can occur in many different organs like the lungs, colon, urine bladder or ureters.

The diagnostic analysis of this disease is often difficult. Thus, for clinical classification and staging more information about the location, expansion and adhesion of endometriosis lesions are needed. In the following chapter the different hypothesis according etiology will be described in detail (1,2).

1.1.1 Etiology

Since Rokitansky 1860 first described the disease, it is of high gynaecological interest, due to its massive occurrence in women. Therefore, a lot of research was carried out to elucidate the pathogenesis of this disorder and Sampson and Meyer postulate the two main theories according the development of endometriosis. These theories are highly discussed and are of scientific interest until now. The first hypothesis of Sampson deals with the retrograde menstruation and a second one with immunologic and vascular/lymphatic spreading. Alternatively, Meyer elucidated the metaplasia theory which also belongs to one of the main theories for the genesis of endometriosis that were still discussed (1,2).

1.1.1.1 Retrograde menstruation

Retrograde menstruation is also known as the transplant-theory of Sampson, and it is still the best-researched and accepted theory. It postulates that during menstruation vital endometrial cells migrate through the tubes into the abdomen and spread there. Researchers have found vital endometrial gland cells in the menstrual blood which brought them to the hypothesis that these cells could function somewhere else.

Furthermore, it was found that endometriosis mainly manifests in women with hypermenorrhoea or decreased cycle lengths (1). On the one hand menstrual disorders could result in endometriosis, but otherwise also endometriosis itself could trigger abnormalities in the menstrual cycle. This fact would also support Sampson's theory of retrograde menstruation (2). Furthermore, some papers also declare the possibility for endometrial cells to link to the peritoneal tissue which also strengthens this theory (3). In the study of Aoki and co-workers it was demonstrated, in a mouse model of endometriosis that vital endometrial cells can attach and spread in the peritoneal tissue (3,4). Apart from that this theory is not able to explain the presence of endometriotic lesions in women without endometrial anomalies or abnormalities of menstrual bleeding.

Scientifically, retrograde menstruation is accepted to appear in most women but only a few of them suffer from endometriosis as it was shown by Liu and colleagues in 1986 (4,5). This

raises the question of whether retrograde menstruation could be the reason for the development of endometriosis or just appears as a physiological phenomenon in women while the pathophysiology of endometriosis has another origin. Since just a few women develop endometriotic lesions as consequence of retrograde menstruation the reason for that phenomenon is not clarified yet and also the influence of immunological processes in the pathology of endometriosis is discussed (1).

1.1.1.2 The vascular or lymphatic spreading theory

In "The American Journal of Pathology" Sampson published in 1927 an additional theory for the formation of endometriosis based on his findings concerning uterus shapes. Therefore, he filled uteri that were removed during different stages of the menstrual cycle with a special mixture of gelatine and bismuth subcarbonate or barium sulphate (6). Sampson found that in some cases the mass he injected "*escaped into the venous sinuses of the uterine wall and through the uterine and ovarian veins*" (Sampson, 1927). This phenomenon usually happened, if the uterus was removed during the menstruation of the women. These observations led Sampson to the assumption that endometrial tissue can be distributed through the venous and lymphatic system during menstruation (1,6). Along with Sampson's observation and the fact that cancer cells can metastasize and spread into other organs, this theory should be taken into consideration as possible hypothesis for the development of endometriosis. Even if accepting this theory will not explain the fact, that in some women the distributed cells would graft in other organs (2).

1.1.1.3 Metaplasia theory

Meyer's metaplasia theory rises the idea of coelomic epithelium metaplasia. In this theory it is assumed that the coelomic epithelium is constantly experiencing changes in the environment (2). Some of these changes such as different oestrogen levels may have an influence on the pluripotent coelomic epithelium which may result in metaplasia and what is more they would lead to endometriotic lesions (2). The metaplasia hypothesis is based on the fact that it is only provoked by different environmental influences. It does not reject

the transplant-theory, to the contrary it even completes this theory of Sampson. Some questions that come up in the transplant-theory could be answered with the metaplasia-theory, e.g. the occurrence of endometrial lesions in women without uteral endometrium or even the appearance of such lesions in men (2,7–9).
Therefore, the metaplasia theory supports the transplant-theory and should be considered as an important theory (2).

In conclusion all theories mentioned above should be taken seriously and may be considered as the right way in understanding the pathogenesis of endometriosis. All theories seem to be legit and especially the theories interconnected with each other should be of high interest. Especially the influence of the immune system in these theories should be examined.

In consideration of this thesis the role of the immune system in these theories should be highlighted. On the one hand the immune system may have an important impact on the implantation of the endometrial cells, which spread during retrograde menstruation (2). However, it is not clarified yet if changes in immune cells are the trigger for implantation or just the result of different changes.

1.1.2 Clinic

1.1.2.1 Prevalence

The Prevalence of endometriosis varies greatly due to the unspecific symptoms and the difficulty of diagnosis (2). On average five to ten percent of women between menarche and menopause may be affected by endometriosis. However, the estimated number of unreported cases would be much higher and especially in infertile women this number exceeds the 50 percent mark (1).

1.1.2.2 Symptoms

The possible symptoms of endometriosis are of high variety, which is a further reason for having many patients undiagnosed.

The main symptom is painful menstruation also known as dysmenorrhea. Often dyspareunia, genital pain during intercourse, is present. About fifty percent of women with endometriosis are affected by this symptom (1). Another main problem is infertility, which often leads to the diagnosis and occurs in almost half of patients with endometriosis. Furthermore, changes of menstruation such as extended bleeding or spotting before menstruation often occur and also changes in defecation and urination like pain or blood can appear (1).

Especially infertility, dysmenorrhea, abdominal pain and menstrual disorders are highly suspicious for endometriosis and are known as the cardinal symptoms (2).

1.1.2.3 Diagnosis

The clinical symptoms primary lead to the suspicion of endometriosis. First of all, a careful clinical examination with a detailed anamnesis should be performed. Nevertheless its result depends on the manifestation and localisation of the endometriosis isles and therefore the results vary vigorously in their validity (2).

The next step of diagnosis includes imaging procedures. The three possibilities of interest are ultrasound, computed tomography (CT) and magnetic resonance (MR) imaging. Even if none of them is proving endometriosis, they can help in the process of finding a diagnosis. Especially because of the high invasiveness of the laparoscopy, the only proving instrument, imaging procedures should be seen as a supportive step (2).

Ultrasound images help to distinguish between solid and cystic structures. Furthermore, an approximate localisation can be detected. This examination is cheap, easy to perform and of no risk for the patient, as a result it is done frequently. However, ultrasound cannot differentiate haemorrhagic cysts from endometriosis lesions.

The computed tomography otherwise has no great benefit for the patient in finding a diagnosis. Additionally, it leads to high radiation exposure and is very expensive. Thus, it is rarely performed (2).

The MR imaging is also very expensive and, with one exception, of no diagnostic interest. Just in adenomyosis uteri it has a high sensitivity and specificity even in comparison with ultrasound and the gold standard, laparoscopy (2).

As mentioned before, laparoscopy with taking histologic samples is the only way to ensure the diagnosis (1,2). Hence, laparoscopy is the gold standard in making the diagnosis of endometriosis. Laparoscopic results lead to further information about staging, location and activity level. Due to the invasive procedure of laparoscopy, surgical endoscopic treatment is often performed at the same sessions nowadays (1). However, recently some guidelines tend to prefer empiric therapy over laparoscopy partly because of the lack of coherence of severity seen in laparoscopy compared to symptoms (1,10). Furthermore, laparoscopy itself is challenging especially in non-heterogenous lesions or hidden lesion locations therefore some guidelines only mention laparoscopy in treatment algorithms, if fertility is the main focus (10).

1.1.2.4 Therapy

Due to the variety in clinical presentation and the unexplained etiology of endometriosis there is no standardised therapy. Therapies are rather adapted individually to the patients´ needs. Therefore, different goals of treatment exist, which are partly treated completely different. Especially the treatment of infertility and the existing desire to have children determine the path of treatment (2).

Goals of treatment are normally analgesia, treatment of infertility, treatment of ovarian endometriomas and endometrial lesions beyond pelvis minor and to maintain recurrence freedom as long as possible (2).

Basically, the treatment possibilities are distinguished in pharmacological options and the surgical therapy and in certain cases a combination of both therapeutical approaches is applied. (1) As there is no profound cure for endometriosis by the current state of scientific knowledge the treatment possibilities are rather symptomatic than curative (2).

1.1.2.4.1 Pharmacologic therapy

Analgetics

The administration of painkillers is primarily for symptomatic pain therapy. Due to dysmenorrhea being one of the main symptoms, pain treatment is very important (1). Increased prostaglandin concentrations in endometrial lesions assumed to be part of the trigger for pain. Therefore, prostaglandin synthesis inhibitors are mainly used to manage pain (2). However, the intake should happen before the symptoms even arise. Mostly this is no problem because pain occurs in a cycle dependent manner (2).

Hormonal treatment

The concept of the pharmacological endometriosis therapy is based on the recognition that endometriosis symptoms decrease during pregnancy or menopause. The improvement of symptoms refers to the hormonal changes (1). The aim of the pharmacologic therapy is to induce similar hormonal balance changes. Mainly these changes lead to a suppression of the ovarian function and atrophy of the endometrial tissue (1).

The knowledge of the female hormonal regulatory circuit is important for understanding the following therapy concepts. The hypothalamic gonadotropin-releasing hormone (GnRH) induces the release of follicle-stimulating hormone (FSH) and luteinising hormone (LH) in the pituitary gland (11). FSH leads to oestrogen production, which leads to endometrial proliferation. LH brings about the development of the corpus luteum, which produces gestagens. Gestagens cause endometrial evolution and decrease womb contractility. Furthermore, gestagens and oestrogens inhibit through negative feedback mechanisms FSH and LH production (11).

Gestagen therapy

Gestagen-monotherapy is a second-line therapy, when combined oestrogen-gestagen hormone therapy, which is considered as the first-line therapy, failed (1,2,12). Lately some authors support gestagen-monotherapy as a first-line therapy, it should be beneficial compared to combined-hormone therapy (12). However current studies data are

conflicting and the advantage of gestagen monotherapy compared to other hormonal therapies is unproven.

The reduced oestradiol level during gestagen therapy leads to endometrial decidualization but with little influence on the endometrial lesions. Additionally, amenorrhoea is a side effect of long application of gestagens. Usually, long-term therapy is required for what reason depot treatment is preferred (2). Furthermore, gestagens cause pain relief and compared to combined therapy the negative side effects of the oestrogen part are avoided (1).

Combined oestrogen-gestagen therapy

The combined oestrogen-gestagen therapy like in contraceptive pills leads to ovarian hormonal suppression. However, initially it is stimulating the endometrium, afterwards it leads to amenorrhoea (1). The main effect is inhibition of ovulation and decidualization or atrophy of lesions leading to necrobiosis (2,10). Due to the severe side effects high-dose or long-term therapy with contraceptive pills is obsolete (1). There exist different ways of application that include the continual or cyclic application. The continually application has a better outcome (2).

In 2008 a placebo-controlled, double-blind, randomized trial confirmed that oral contraceptive pills lead to improvement of dysmenorrhea and size reduction of endometriomas larger than 3 centimetres (13,14).

GnRH agonists

Gonadotropin-releasing hormone agonists are considered as second or even third line therapy. They inhibit ovarian steroidogenesis and are as effective as gestagen monotherapy and combined oestrogen-gestagen therapy (15).

Main target of the GnRH agonists therapy is reducing serum oestradiol levels, which leads to endometrium and endometrial lesions atrophy. Initially, the application leads to a short boost with intensification of symptoms (2). The application form is challenging because oral application is not possible due to high reduction in the gastro-intestinal-system. Depot injections proved itself in practice, but individual serum levels vary greatly and therefore

serum levels should be checked regularly. The main side effect of low levels of oestradiol is the loss of bone density in long-term treatment. Long-term therapy, over six months, is not recommended due to the serious side effects (2). A certain indication for GnRH therapy is presurgical lesions size reduction and moreover, it is considered as a therapy supporting in vitro fertilization in women with endometriosis (1). Otherwise, there are some studies that disprove GnRH therapies' benefit concerning supporting in vitro fertilization. A review of Georgion et al. summarised the data of different studies that found no difference comparing life birth rate between IVF with prior GnRH therapy and without (16).

Levonorgestrel-releasing intrauterine system

Levonorgestrel-releasing intrauterine systems, mostly Mirena, are used in endometriosis therapy lately. However, this therapy is an off-label use but some studies show the benefit of IUS especially in pain relief therapy (2,13).

1.1.2.4.2 Surgical therapy

The most important basis of decision-making for the procedure of surgical therapy is the therapeutic target. Principally, there are two main targets either analgesia or the recovery of fertility (2). Additionally, surgical therapy will be chosen, if there are adhesions, which should be loosen as pharmacological therapy has no effect on them, or for endometriosis lesions and cysts resection (1).

If fertility is the priority a very careful surgical method would be chosen with minimal resection to secure the physiological paths and with the aim to destroy as little healthy tissue as possible (1,2).

However, if pain relief is the main target, given that there is no wish for children, a more radical procedure will be executed. In this case the endometrial lesions will be resected as far as possible. But it should be kept in mind that even the radical method is not completely curative (2). Even after removing the lesions, new lesions can form of the subperitoneal tissue (17,18). Furthermore, the recurrence rate for surgical therapy varies between 20 and 50%, which is very high for such an invasive procedure (2). A study from 2009 even came to the conclusion that one year after surgical therapy nearly half of the patients needed

analgesia or hormonal therapy, and even in about 25 % of cases a revision of surgery is needed (17,18).

1.2 Immune system

The immune system is a complex system of vital importance for body functions. To provide protection against many different types of pathogens many different parts are working together. The immune system can either be divided into the humoral part and the cellular part or into the innate and the adaptive immunity. Basically, the main function of the immune system is the defence of the body against diseases and pathogens. Pathogens are small particles causing diseases. The most important function is the ability of the immune system to distinguish between healthy tissue and possible pathogenic tissue. It is a very complex system and therefore, it is attackable and not resistant to changes. Aside from that, many small parts must work together to ensure proper function. (19).

Leukocytes play a major role in the innate as well as in the adaptive immune response. They are white blood cells, mostly developed in the bone marrow. Generally, matured immune cells leave the bone marrow and migrate into the peripheral tissues. This is why they could not only be found in the tissue but also in the blood circulation and the lymphatic system (20). However, some develop already during embryonic development and migrate into tissues before birth (20).

The pluripotent hematopoietic stem cell is the progenitor cell for all blood cells. Two types of stem cells, the lymphatic and the myeloid ones, develop from this type of stem cell. The lymphatic progenitor cell can further develop into B-lymphocytes, T-lymphocytes, Natural-Killer-cells and innate lymphoid cells (ILC). Alternatively, the common myeloid progenitor cell generate mast cells, macrophages, myeloid dendritic cells and granulocytes (20,21).

1.2.1 Innate immunity

The innate immune response is the unspecific one. It includes the first line defence against microorganisms, foreign substances and tissue damage. This response requires no previous

contact with a pathogen or other noxae to react correctly. Even repeated contact to the same pathogen/noxa will not lead to an increased response (22).

Barriers are of utmost importance for the proper function of the innate immunity. Mechanical barriers like intact skin and mucous membrane barriers are important to protect the body against invasion of microorganisms (20). Additional, biochemical barriers exist, which produce fatty acids and bactericides to provide further protection (22). The humoral part of the innate immune system includes the complement system as well as the coagulation system. (22). Here, the complement system is composed of thirty different types of plasma proteins cooperating with antibodies (20).

The main cellular part of the innate immunity are phagocytes like monocytes, macrophages, dendritic cells and neutrophil granulocytes as well as mast cells, basophil and eosinophil granulocytes and natural-killer cells (22).

The innate immune cells present special receptors that are called pattern recognition receptors (PRR). These receptors are used by the immune cells to detect and connect special molecular structures on the surface of pathogens. These structures are called pathogen associated molecular patterns (PAMP), which are different to body structures (22–24). Furthermore, another type of these molecular structures exists, which is called damage associated molecular patterns (DAMP). DAMP are found as endogen molecules on stressed and necrotic cells. If PRRs bind to PAMP or DAMP a signal transduction cascade get started, which leads to synthesis and secretion of antimicrobic peptides, cytokines and chemokines. These mediators attract and activate leukocytes, leading to an inflammatory reaction (22–24).

In the following the immune cells of the innate immune system will be described in detail.

1.2.1.1 Phagocytes

As already mentioned above macrophages, monocytes, dendritic cells and neutrophil granulocytes are phagocytes. They incorporate PRR mediated pathogens and foreign material by phagocytosis and endocytosis. In further consequence, these particles are enzymatically decomposed by the phagocyte. Through the phagocytosis process these cells

produce cytokines, enzymes, antimicrobic peptides and reactive oxygen species, which are toxic to microbes (22).

Neutrophil granulocytes are specialised in defending the body against bacteria and fungi. Monocytes for example migrate into tissues and turn into macrophages or dendritic cells. These types of cells belong to antigen-presenting cells, that present antigens on their surface. This antigen representation is of major importance for the development of the adaptive immune system (22).

1.2.1.2 Mast cells/basophil granulocytes

Basophilic granulocytes are localised predominantly in the peripheral blood and their lifespan is quite short. On the contrary, mast cells live quite long and are found in the tissue. Upon activation they produce proinflammatory mediators like chemokines and cytokines there, leading to attraction and migration of inflammatory cells (22).

These two cell types are stimulated by pathogens as well as the complement system to release their granules including amines, proteases and cytokines. Furthermore, they express receptors for a part of IgE-antibodies (22).

1.2.1.3 Eosinophil granulocytes

Eosinophil granulocytes are responsible for parasite destruction by binding, with their IgE-receptor, to the IgE-molecules on the surface of the parasite. They also have granules with enzymes, toxic proteins, cytokines and proinflammatory mediators. They are activated by PRR binding or through interleukin (IL)-5, which lead to granule release and production of reactive oxygen species to kill the parasites (22).

1.2.1.4 Natural-killer cells

The main target of natural-killer cells (NK-cells) are stressed body cells. Natural-killer cells have activating as well as inhibiting receptors. Ligands, on stressed cells, bind to the

natural-killer cells surface receptors and activate them. Due to activation cytoplasmatic granules containing perforins, proteases and tumour necrosis factor α (TNF-α) are released and leading to apoptosis of the target cell. Furthermore, NK-cells produce interferon-γ (IFN-γ), which subsequently activates macrophages (22).

To ensure that NK-cells do not destroy healthy body cells, they express inhibiting receptors called killer cell immunoglobulin-like receptor (KIR). These receptors bind to the major histocompatibility complex I (MHC-I), which is found on healthy cells. If this binding occurs the natural killer cell is inhibited and will not attack the healthy cell (4,22).

1.2.1.5 Proinflammatory mediators

The proinflammatory mediators mentioned above include cytokines like IL-1, IL-6 and TNF-α, chemokines like IL-8 and inflammatory lipid mediators like leukotrienes (LT), prostaglandins (PG) and platelet activating factor (PAF) (22).

Cytokines are proteins released from cells, affecting surrounding cells, if they have specific receptors. Basically, their main target is to enhance effector mechanisms. This is important for the communication and interaction of different immune cells in pathogen destruction. At the same time chemokines are secreted proteins as well, but they act as chemoattractants for cells to direct them to certain places like the site of inflammation and enable immune cells to migrate from the vessels to the tissue (20).

1.2.2 Adaptive Immunity

The adaptive immunity is also known as the acquired or specific immune system since its defence against pathogens is highly specific. The main operating mode consists of B- and T-Lymphocytes and antibodies. The cellular part, B- and T-Lymphocytes, is responsible for contact with antigens and the formation of specific antibodies, which represents the humoral part of the acquired immunity. Its characteristics are diversity, specificity to antigens and the so-called immunological memory (22).

B- and T-Lymphocytes function is based on the ability to detect antigens with receptors on their surface, which is known as the antigen specificity. These receptors vary massively in their structures, which is important to detect many different antigens, this is the diversity mentioned above. The immunological memory is the ability of the adaptive immunity to react in a faster and stronger way to a recurrent infection. This is possible due to the activation of memory cells formed during the first contact with the antigen (22).

Of major importance is the exact function of this system. To protect the body of being attacked by these cells, safety systems exist which lead to elimination. This elimination of autoreactive cells normally proceeds during ripening processes. If they are missed in this first process, they usually are inactivated in the peripheral tissue. Dysfunction of this elimination processes end up in allergies or autoimmune diseases (22).

2. Materials and Methods

Literature research

The type of this thesis is a literature research with the aim to compare studies to the influence of the immune system on the pathogenesis of endometriosis. Therefore, databases and journals, such as PubMed, OvidSP, UpToDate and Google Scholar have been searched. The search terms endometriosis, immune system, immune cells, macrophages, mast cells, eosinophiles, neutrophils, basophils, natural killer cells, NK-cells, dendritic cells, DCs D-cells, B-lymphocytes, B-cells, T-lymphocytes and T-cells were used. Additionally, these search terms have been expanded by keywords like pathogenesis, guidelines, definition, therapy, surgical therapy, medical therapy, interventions, etiology, etiology theories, angiogenesis, VEGF, diagnostic and complications. Variations in spelling were also taken into account. The literature research was conducted between June 2019 and February 2022 and books, articles, reviews and guidelines in German or English, published until February 2022 have been used as source of information. With this collected data an overview about endometriosis itself and the influence of the immune system has been compiled. Mendeley has been used as a literature management program and the literature sources in Vancouver Style quoted.

Data analysis

The studies were selected by accessibility and actuality. Furthermore, only studies in English or German have been used. Reviews have been considered as well, but the primary literature summarised in these reviews have been searched and used for data analysis. The studies have been divided into the different immune cell types. The different study results to one immune cell type have been compared in separate chapters. The analysis focused on the effect of the immune cell types, the number of study population, the actuality and the results. Especially, the study design construction and result finding has been considered.

The different study designs with different types of study populations have been of special interest. Due to the studies comparing different types of populations, for example women with and without endometriosis or women with severe and mild endometriosis or women

with endometriosis and women with other gynaecological diseases, comparing the studies was complicated. A difference in study samples, for example peritoneal fluid, endometrial tissue or blood, has been existing as well. For which reason, comparisons have been made between studies with the same populations and samples, if not possible the difference was outlined.

3. Results

3.1 Natural killer cells

The basic functionality of natural killer cells is outlined above. However, for understanding of NK-cell alterations it must be elucidated in more detail. The role of natural killer cells in pathogenesis of endometriosis is highly discussed in several studies. Especially, their absence in destroying ectopic endometrial tissue is of high interest (4,20).

Natural-killer cells are part of the innate immune system and can detect cells that should be destroyed due to their risk for the bodies' functions. The ability to distinguish between infected and non-infected cells is of major importance for NK-cells. Therefore, they have different receptors that can be activated or inhibited for the protection of the body (4,20). The main functionality of NK-cells consists of three different recognition mechanisms: the so-called i) "induced self", ii) "missing self" and iii) "modified self" mechanisms that react to the presence or absence of various effector or cell surface molecules. Thus, the receptors are able to recognise cell surface molecules, which can be altered due to stress or infection. The "induced self" recognition as well as the "modified self" recognition implies that receptors detect target cells through surface molecules triggered by metabolic stress (like HLA-E ligands). At the same time NK-cells induce the release of cytotoxic granules when specific cell surface molecules like MHC-I are less expressed or completely missing on target cells in consequence of infections or tumours. This phenomenon is then called the "missing self" detection (4,20). Additionally, NK-cells interact with effector molecules by releasing cytokines, especially IFN-γ and cytotoxic substances (4).

Alterations in target cells, NK-cell subtypes and the local environment define the type of NK-cell reaction (4). Especially changes in frequency, cytotoxic activity, expression of receptors and phenotypes are discussed to have impact on endometriosis.

3.1.1 NK-cell frequency and cytotoxic activity

As part of the alterations in destroying the ectopic endometrial tissue, NK-cell frequency and cytotoxicity was discussed to play a role (4,25,26). Many different studies looked for changes of NK-cell numbers in peripheral blood and peritoneal fluid (27,28,37–39,29–36).

However, the majority of them found neither a difference in peripheral blood nor in peritoneal fluid compared to women without endometriosis (27–34). On the contrary, Szyllo and colleagues demonstrated in 2003 that NK-cell frequency was decreased in peripheral blood and it was increased in the peritoneal fluid of women with endometriosis compared to healthy ones (39). Moreover, the article refers to a study of Oosterlynck and co-workers, who have also found decreased NK-cell numbers (35,39). Also other working groups found differences in NK-cell frequency, like the groups of Tariveridian and Kang, who found diminished numbers of NK-cells in the peritoneal fluid as well and what is more the decrease shown by Kang and colleagues was a significant one (36,37). Dias and colleagues on the other hand found increased NK-cell numbers in peripheral blood, however interestingly observing higher numbers in women with advanced endometriosis (38).

Furthermore, just a few papers focused on natural killer cell appearance in endometrial tissue whether ectopic or in the uterus. They either found no significant difference (40), or decreased frequency in ectopic endometrial tissue (17,25) or increased in eutopic endometrial tissue (41).

The cytotoxic activity of NK-cells may be important for endometrial tissue clearance. Alteration in cytotoxicity of these cells have been reported multiple times. Most studies focused on reaction against K562 cells, which either found diminished cytotoxic activity in peritoneal fluid (42–44), peripheral blood (28,35,42) or no differences (44,45). K562 cells are a tumour cell line, which is used as a target cell for natural killer cells (45,46). Just a small amount of studies exist that explore cytotoxic activity against endometrial cells, which may be more meaningful regarding endometriosis (4,25). One of these rare studies on endometrial cells was performed by Oosterlynck and colleagues, which found a diminished cytotoxicity against the endometrial cells (27). This leads to the assumption that women with endometriosis are incapable to destroy endometrial cells. Furthermore, they found an increased loss of function in relation to higher phases of the disease (27). However, the study also researched the use of heterologous lymphocytes in the lysis of endometrial cells, which was also diminished in higher phases of the disease, leading to the question if endometrial cells may change in women with endometriosis so that NK-cells have difficulties to interact with them (27).

3.1.2 NK-cell subpopulation, phenotypes

Natural killer cells can be divided into two major subpopulation groups, which differ in their cell surface molecule the so-called cluster of differentiation expression. Thus, they are defined as $CD56^{bright}$ $CD16^{-}$ and $CD56^{dim}$ $CD16^{+}$ NK-cells. A few more markers and receptors, they differ in, exist (47). These subtypes differ in their function, i.e. $CD56^{dim}$ $CD16^{+}$ cells are of high cytotoxic potential whereas $CD56^{bright}$ $CD16^{-}$ produce higher amounts of cytokines and chemokines like IFN-γ and TNF-α. Furthermore, the majority of NK-cells in peripheral blood comprise of $CD56^{dim}$ $CD16^{+}$ cells (47). It is assumed that $CD56^{bright}$ $CD16^{-}$ cells are likely to be the less differentiated cell line, which can mature into $CD56^{dim}$ $CD16^{+}$ and gain cytotoxic potential (48). Aside from that, it is supposed that the location of the NK-cells has an effect on their function and phenotype (47,49,50). Regarding endometriosis especially uterine NK-cells could be of interest. The endometrial NK-cells are of $CD56^{bright}$ $CD16^{-}$ subtype and differ from the ones in the peripheral blood, because they are granulated. These uterine NK-cells (uNK) are assumed to play an important role during pregnancy in implantation and placentation. Furthermore, they are relevant in spiral artery remodelling (49,50). Spiral artery remodelling is seen to be important for forming endometriosis lesions too, which can point out a connection to endometriosis.

3.1.3 Variety in receptors and ligands

Many different receptors expressed on NK-cells, which stimulate or inhibit this cell type, exist and are important for recognising different ligands for interaction. Some of these vary in endometriosis patients. One of these receptor families is called killer immunoglobulin-like receptors, these mainly interact with human leukocyte antigen (HLA) ligands (25). For example the KIR2DL1 receptor, which is an inhibiting one, was increased in peritoneal fluid and peripheral blood of women with endometriosis (32,51). Whereas the KIR2DL2 receptor showed no difference in comparison with healthy women (31,32,51). Furthermore, another ligand, HLA-G, interacting with a KIR receptor namely KIR2DL4, was investigated and assumed to have no difference in women with and without endometriosis. However, the expression of HLA-G ligands was only seen in women during menstrual phase (52). A few different KIR receptors, like KIR2DL3, KIR3DL1 and KIR3DL2, as well as the KIR2DL4

ligand HLA-G would be of interest for the understanding and/or a possible therapy of endometriosis, but current studies are missing (25).

As already mentioned, natural killer cells detect MHC-I class molecules on body's own cells to prevent them from being destroyed. Since this could be a reason for diminished NK-cytotoxicity against ectopic endometrial cells, some studies focused on this relation. One research team actually found an increased expression of MHC-I in patients with endometriosis (53).

Furthermore, another study found no difference in the mean serum concentration of HLA-1 from healthy women and endometriosis patients overall disease stages. However, comparing endometriosis patients according to disease stage they found higher numbers of the molecule in the serum of patients with lower disease stages compared to women with advanced endometriosis (54). Whereas another group found diminished HLA-1 numbers in the sera of women with endometriosis compared to a healthy control group (55). This may require further studies.

Another type of receptor is called CD94/NKG2. It includes a few subtypes called A, C, D, E and F of whom A, C and D were of interest in combination with endometriosis. (20). NKG2A for example, known to be an inhibiting receptor, was found to be increased in peritoneal fluid of women with the disease (20,33). However, the expression of this receptor showed no difference in peripheral blood (33). The subtype NKG2C works as an activating receptor and was seen to neither be increased or decreased in endometriosis patients (20,33). The NKG2D receptor structure is different to the other ones and binds stress-induced molecules like MIC-A, MIC-B and RAET1, it is known as an activating receptor as well (20). Guo and colleagues found diminished numbers of NKG2D in endometriosis patients (56). Another study group found increased MIC-B and MIC-A in peritoneal fluid of women with endometriosis, although MIC-A was only significantly increased in higher stages of the disease (57). The increase correlated with the severity of the disease. Furthermore, the authors thought of an downregulation of NKG2D due to the increased MIC-A and MIC-B (57). This is just an assumption and would need further confirmation.

3.1.4 Influence of cytokines on natural killer cells

Cytokines are molecules, important for cell communication. They are secreted by cells and through binding specific receptors they lead to an interaction (22). Some of these cytokines are discussed to have influence on endometriosis. Transforming growth factor (TGF)-β was seen to be increased in the peritoneal fluid and serum of women with endometriosis. Although, the increase was much higher in women with higher stages of the disease. Furthermore, the increase in the peritoneal fluid was much higher than in the serum of the patients (58). Another study also found increased TGF-β1 in peritoneal fluid (56). TGF-β was also discussed to may inhibit NK-functions, like cytotoxicity and diminished expression of NKG2D, which is a stimulating receptor (20,25,56). This study group analysed the possible connection between increased TGF-β1 and decreased cytotoxicity of NK-cells. Therefore, they incubated peritoneal fluid with antibodies against TGF-β1 and interestingly found an increase in cytotoxicity of NK-cells (56). Due to this result an impact of TGF-β on NK-cells seems to exist. Furthermore, their influence on NK-cell cytotoxicity, which would be important in endometriosis, could be pivotal for the survival of ectopic endometrial cells.

Another cytokine of interest is interleukin-6, which was found to be increased in just peritoneal fluid (37) or peritoneal fluid and serum of women with endometriosis (58). The study group, which found the increase only in the peritoneal fluid linked it to an increase in macrophages, which they found in the same study, producing most of IL-6. Furthermore, they showed a correlation between IL-6 and cytotoxic activity of NK-cells by improving the cytotoxic activity by using IL-6 antibodies in peritoneal fluid samples (37). Furthermore, this samples showed increasing granzyme B levels, which is a protease important for initiating apoptosis. This leads to the assumption that IL-6 suppresses granzyme B expression (17,32).

Interleukin-15 is another cytokine of studies interest in endometriosis research because it was seen to be increased in patients with endometriosis. Moreover, this study group found an diminishing effect of IL-15 on granzyme B, NKG2D, which is an activating receptor, and on NKp44, a natural cytotoxic receptor (59). They even found data suggesting IL-15 has an impact on the growth and invasion of endometrial stromal cells (59).

Additionally, interleukin-10 and interleukin-4 were elevated in women with endometriosis (60,61) or of no difference compared to a control group in two other studies (62,63).

Interleukin-10 is known to have a diminishing effect on NK-cell function (20,22,60). Hence, if these interleukins are elevated in endometriosis patients, their NK-cell function reducing effect could create an endometriosis-promoting environment.

Interleukin-12B, also known as p40, a subunit of IL-12, was also seen to be increased in the peritoneal fluid of endometriosis patients (64). IL-12 has an activating effect on NK-cell function which could be impaired by IL-12B (25,64).

Interferon-γ (IFN-γ) is a cytokine responsible for initiating cell apoptosis. This function may be inefficient towards endometriotic cells (65). IFN-γ was seen to be increased (62), diminished (61,66) or of no difference (63,67) in the peritoneal fluid or of no difference in the serum (63) of women with the disease. The different results could suggest that this cytokine has no influence in endometriosis. An increased amount of IFN-γ could indicate more apoptosis or that IFN-γ is not effective towards endometriotic cells whereas a lack of IFN-γ could imply a absent cell apoptosis for endometriotic cells and therefore an unphysiological survival of that cells. However, the function for cell apoptosis in endometriosis might play a role, although a possible non-response of endometriotic cells to this function would underline the unimportance of IFN-γ in endometriosis.

Furthermore, tumour necrosis factor-α (TNF-α) also known to be important for apoptosis and anti-inflammatory response (22) was found to be increased (62,66) or of no difference (67) in peritoneal fluid of women with endometriosis. If TNF-α is increased it could help prevent endometriosis development, due to its ability to destroy cells. On the other hand the anti-inflammatory character of TNF-α could lead to decreased response against endometrial cells and promotes cell survival.

These cytokines mentioned above may play a role in alteration of NK-cell function, especially TGF-βs study data reveals a pivotal role of it. Unfortunately, the present state of studies is not detailed enough and should be of more interest in the future.

3.1.5 Conclusion

The role of natural killer cells in endometriosis progression may be important. Alterations of NK-cells have been confirmed, leading to diminished destruction of endometrial cells.

Whether this inability is due to the varied NK-cell frequency or altered cytotoxicity remains unknown. Studies focused predominantly on cytotoxicity due to the different results regarding the frequency of NK-cells (4,25,26,68). Although the studies regarding cytotoxicity against endometrial cells would be of interest, they are rare. The results of diminished cytotoxicity against endometrial cells are of high potential. Especially the receptor variability in endometriosis patients, like diminished activating and increased inhibiting receptors, lead to the assumption of an important role in endometriosis. Furthermore, cytokines interacting with the immune environment and NK-cells may be important. Especially TGF-β is of major interest, due to the findings that cytotoxicity was increased after TGF-β-antibody treatment (4,25,26,68).

In conclusion NK-cells seem to be important for endometriosis, however, there is no information about whether these alterations lead to endometriosis or if the disease leads to these alterations. But the data suggest that NK-cells are at least important in endometriosis progression. Certainly, one review found interesting information about studies in mice models. In these studies, they found on the one hand implantation of the endometrial tissue independent on natural killer cells. On the other hand they found permanent implantation of ectopic endometrial tissue when inhibiting NK-cell activity (3,4).

Furthermore, the differences in study data complicates the evaluation. These differences may result from low patient populations. Thus, further studies would be important for more detailed results.

3.2 Macrophages

Macrophages contribute to inflammatory responses as an important part of the immune system. They act as antigen-presenting cells and interact with T-cells. This collaboration consists of exchanges of different cytokines, which lead to pathogen destruction and alterations (20,22).

3.2.1 Subpopulation and phenotype

Macrophages can be classified in M1 and M2 macrophages. While M1 macrophages are predominant especially in acute inflammation leading to a pro-inflammatory environment, M2 macrophages act in a opposite way by generating an anti-inflammatory milieu and contribute to angiogenesis, wound healing and homeostasis (69,70). Moreover, M1 macrophages have a highly developed antigen-presenting character and special cytokine production, like IL-12, IL-23 and nitric oxide synthase (NOS), leading to pro-inflammatory reactions. On the contrary M2 macrophages produces IL-10, Vascular endothelial growth factor (VEGF) and TGF-β and are important in tissue repair and tissue remodelling as well as immunosuppression and immunoregulation (71).

This strict classification, however, can just be made in vitro. In vivo and especially in disease the macrophages are of more complex phenotypes. Furthermore, a special ability of macrophages is discussed. They could be able to adjust their gene expression and thereby adapt their phenotype with the help of different mechanisms which remain unclear (72,73). These mechanisms would be of great interest in disease pathogenesis, but further information is required.

Another study claimed that macrophages change their phenotype gradually. Whereas the macrophages were of pro-inflammatory phenotype with NOS production and MHC-II surface molecules at the beginning. Within two weeks it was observed that the phenotype changed towards a tissue remodelling one (73).

These findings and the fact, that some research groups distinguish in M1 and M2 and some do not, make it difficult to compare the results in the following.

3.2.2 Frequency and phagocytic activity/ability

Studies regarding the frequency of macrophages in endometriosis are quite different. The number of macrophages in peritoneal fluid (74) and eutopic endometrium (75–77) of women with endometriosis was increased compared to healthy women. On the one hand, these studies distinguished between different cycle phases (75) and on the other hand, the different cycle phases would not be taken into account (76,77). Whereas others

distinguished between the types of macrophages, who then claimed M2 macrophages being dominant in endometrial lesions (26,78). Apart from that, this type of macrophages was seen to be dominant in healthy endometrium and in eutopic endometrium of endometriosis patients this subtype was only found in small numbers (69,77,79).

It is discussed that the enhanced frequency of macrophages could be a result of a dysfunctional so-called macrophage disappearance reaction. Different studies demonstrated that during inflammation this mechanism was absent and led to a higher concentration of macrophages due to increased proliferation (80,81).

Furthermore, the macrophage function, especially the phagocytic ability may be altered in women with endometriosis. The phagocytic activity of macrophages in the peritoneal fluid and lesions was seen to be reduced (82–84).

Moreover, a study of Braun should be mentioned, which compared blood monocytes of women with and without endometriosis and showed a difference in interaction with endometrial cell proliferation. The monocytes, especially macrophages, of women suffering from the disease ameliorate the proliferation of the endometrial cells, whereas the ones from healthy women aggravated it (85).

The mentioned results may indicate an important correlation of macrophages and endometriosis progression and maybe even pathogenesis.

3.2.3 Possible effect in endometriosis

It is already known that macrophages play a pivotal role in the physiological menstrual cycle. They seem to be important in regeneration and proliferation of the functionalis, shedding during menstruation and they have an impact on gland remodelling and angiogenesis, mainly due to their ability to produce VEGF. These abilities may have an impact on lesion formation and implantation as well (73,86–88). A study from Bacci and colleagues supports the theory that macrophages are important in endometriosis. The study group inhibited macrophages of the peritoneum in mice, which resulted in altered lesions. Especially lesion growth and vessel formation were diminished in these altered lesions (78). They also investigated the effect of different macrophages on lesions.

Therefore, they used pro- and anti-inflammatory as well as non-polarized macrophages. Through this investigation they observed a diminishing effect of pro-inflammatory macrophages on weight and architecture of the lesions (78). Whereas the anti-inflammatory macrophages led to opposite results, raising up the assumption that anti-inflammatory macrophages could be important for lesion formation and growth (78).

Furthermore, Capobianco and colleagues found $Tie2^{+}$ macrophages in human and mice endometrium (89). This type of macrophages contributes especially to angiogenesis. The study group selectively reduced these, which resulted in diminished lesion growth. Furthermore, the ability to form new vessels was lost as well as the ability to organise glands (89).

Moreover, another study group observed mice without VEGFR and found differential vascularisation, specifically the number and size of the vessels in the lesions were reduced (90).

These findings may implicate a pivotal role of macrophages in the pathogenesis of endometriosis.

3.2.4 Interaction with stromal cells

Further, the interaction of stromal cells and macrophages should be regarded as well. Chan and colleagues demonstrated an enhanced invasiveness and reproducibility of stromal cells from the endometrium in vitro, if cultured with macrophages from women suffering from endometriosis (91). Another study used ectopic stromal cells, which led to a diminishing effect on the phagocytic ability of macrophages and enhanced viability and proliferation of stromal cells, in relation to the eutopic ones (92,93).

A further research group investigated endometrioma originated stromal cells, which led to alterations in monocytes. In detail, the macrophages changed to a pro-repair phenotype, which facilitated growth of endometrial lesions (94).

These results should be elucidated in more detail to outline the interaction in disease pathogenesis and progression.

3.2.5 Conclusion

Macrophages being important in the physiological menstrual cycle lead to the assumption that they are involved in endometriosis progression or even pathogenesis as well. The changes in frequency and subpopulation of macrophage population in endometriosis patients may be indicative for their role in the disease (69,74–77,79). Furthermore, the pivotal function of macrophages, phagocytosis, was also seen to be reduced, leading to the suggestion that macrophages could not be able to eliminate the redundant endometrial cells (82–84). Additionally, assuming that results of macrophages improving the endometrial cell proliferation are correct, it could explain the proliferation in endometriosis patients and therefore be pivotal in pathogenesis (85). Further studies are required to assure a confirmation of this fact.

Macrophages are able to produce VEGF, which could be an important factor in pathogenesis as well. It is discussed that this production leads to angiogenesis, which further results in lesion formation and implantation (73,78,86–90). Especially the implantation is of scientific interest due to its pivotal role in endometriosis. Proving the assumption of macrophages being important in the implantation process is of major interest and would indicate a fundamental role of macrophages in the pathogenesis, but further confirmation is required (73,78,86–90). As a result, the role of macrophages and their function, in the pathogenesis of endometriosis should be investigated even more.

3.3 Dendritic cells

Dendritic cells (DC) have a special status in the immune system. They originate from the lymphoid cell lines, the plasmocytoids and the hematopoietic or myeloid cell lines, the myeloids. The myeloid subtype is predominant in endometrium and important in endometriosis (26,95).

As antigen-presenting cells, they are involved in the clearance process and have a special position in the immune system as they are the only subtype that can activate naïve T-cells. These cells are categorized by their maturation status, in immature, semi-mature and mature ones (20,22).

The immature dendritic cells act like a guardian against pathogens by endocytosis and phagocytosis of antigens and presenting them on MHC-II molecules. Additionally, they have another special way to present the antigens via MHC-I molecules called cross-presentation. The semi-mature dendritic cells exhibit more MHC-II molecules and produce less proinflammatory cytokines. They can stimulate T-cells to conduct apoptosis or their transformation in regulatory T-cells.
The mature dendritic cells, which are located in the lymph nodes, express more MHC-I and MHC-II molecules and are able to activate naïve T-cells (20,22).

Research results regarding the effect of dendritic cells on endometriosis are absent, which makes it difficult to generalize results in the following part.

3.3.1 Physiologic endometrial DC distribution

A special role of dendritic cells in the normal menstrual cycle and especially during menstruation is discussed. The distribution of DC subtypes in women without endometriosis could be interesting, therefore this distribution is elucidated in the following. However, the amount of data existing is minimal.

Schulke and colleagues focused on the comparison of $CD1a^+$ for the immature subtype and $CD83^+$ for the mature subtype. Overall $CD1a^+$ cell amount was higher than $CD83^+$ cells throughout all menstrual cycle phases in both layers (95). Comparing the two layers, $CD83^+$ was enhanced in the basalis compared to the functionalis during 2, the proliferative and the secretory phase, out of 3 phases. Whereas $CD1a^+$ numbers were only increased in the basalis during the secretory phase (95). Furthermore, immature dendritic cells enhance during the menstrual phase and are assumed to have an impact on endometrial shedding (95,96).

3.3.2 Frequency

Another study by Schulke and colleagues analysed different types of dendritic cells in different stages in the menstrual cycle and compared patients with and without

endometriosis. Therefore, they used hysterectomy samples and analysed the populations of dendritic cells (96). They differentiated two types of dendritic cells $CD1a^+$ and $CD83^+$. As already mentioned before CD1a is a specific marker for immature and CD83 for mature DCs (96).

They found a significant increase of $CD1a^+$ cells in the proliferative phase in the basalis in endometriosis patients compared to control patients, however in the functionalis they found no differences in all cycle phases (96).

The mature subtype $CD83^+$dendritic cells were diminished in both layers in all cycle phases in women with endometriosis. In both groups the $CD1a^+$ and $CD83^+$ DCs did not change cycle phase dependant (96).

The study group compared the number of $CD1a^+$ cells in the basalis and functionalis during secretion phase, which revealed an increase in the basalis compared to functionalis, in either group. $CD83^+$ cells were increased in the basalis in comparison to the functionalis in the patients without endometriosis, whereas in patients with the disease no difference was detected (96).

Interestingly, immature dendritic cells, which normally increase during menstrual phase, were not enhanced in endometriosis patients. Since they are discussed to be important in endometrial shedding this could be affected (26,96).

Furthermore, another result was interesting. Next to lesions the number of $CD1a^+$ cells in the peritoneum was increased compared with the cell number in the distanced peritoneum (96). Besides no $CD1a^+$ cells were found in the peritoneum of women without endometriosis. This leads to the assumption that dendritic cells have an impact on endometriosis, whether if its role is causal or consequence may remain unknown (96).

However, another study reported a moderate but not statistic significant decrease in peritoneal dendritic cells (36).

Furthermore, Maridas and co-workers analysed the frequency of DC subtypes in peripheral blood. They compared different dendritic cell subtypes in women with and without endometriosis. The immature DC subtype was reduced in the control group during menstrual phase. This reduction could not be seen in the endometriosis group. The number

of more mature DC subtype was reduced in both groups likewise. Moreover, the dendritic cells in the peripheral blood were increased during proliferative and secretory phase in women with endometriosis compared to the control group, however the difference was not a statistically significant (97).

3.3.3 Possible effect in endometriosis

It is discussed that dendritic cells have an angiogenic function, like macrophages. This could be important regarding endometriosis. A study by Fainaru and colleagues demonstrated that dendritic cells infiltrate endometriosis lesions and nearly all of them express VEGFR2. VEGF is a factor important for angiogenesis and vascularisation (20,98).

Furthermore, they found many dendritic cells near to new formed blood vessels in their experiment. These findings implicate an impact of dendritic cells in angiogenesis in endometriosis (98).

Moreover, the study group implanted dendritic cells in endometrial lesions in mice to observe a possible difference to mice without DC implantation. The implantation resulted in lesion growth but differences in vessel frequency could not be found (98). However, the vessels in the implantation group were clogged compared with the ones of the control group (98).

These results would implicate an important role of dendritic cells in endometriosis. Two studies searched for further verification and provided different results.

On the one hand, in a study with wildtype mice and transgenic mice the influence of dendritic cell depletion with a toxin was evaluated. This resulted in an reduction of lesion growth in transgenic mice and the number of dendritic cells within the lesions was reduced too (99).

On the other hand, another study with similar conditions showed that the depletion of DCs in a similar transgenic mice lineage resulted in an increased lesion formation, although the number of DCs in the lesions was reduced (100). These different results are interesting and

maybe result from different timespans of toxin injection and examination of lesion formation in mice.

Izumi and colleagues detected a promotive effect of mannose receptor positive dendritic cells on phagocytosis of dead endometrial cells. Furthermore, the mannose receptor positive dendritic cells were increased in endometriosis patients (101). Moreover, the study group was interested in the different expression of dendritic cells when cultured in presence or absence of dead endometrial cells. Especially, IL-6 and IL-1β were increased when cells were co-cultured with dead endometrial cells (101). Interestingly, these interleukins lead to acute-phase protein production and indirect to phagocytosis resulting in inflammation and immune system activation, which could be important in the pathogenesis of endometriosis (20).

3.3.4 Conclusion

First of all, the lack of studies complicates a wide ranged summary and comparisons of existing studies, should be mentioned. Even if there are two studies on one issue, like Pencovich and Stanic, they delivered contrary results. Therefore, the results and this chapter should be treated with caution.

The involvement of dendritic cells in physiologic menstrual cycle and their important part in the immune system explains the expectation of their role in endometriosis and possible effect in pathogenesis (95,96)

The alterations in frequency of different types of dendritic cells in endometriosis lead to the assumption of endometriosis being in correlation with DCs somehow. Particularly, the reduction of mature dendritic cells in women with endometriosis raises the question of ineffective maturation. Of further interest is the finding of immature dendritic cells in the peritoneum only in the immediate vicinity of lesions (96).

The increased perivascular density of dendritic cells and their enhanced VEGFR2 production, an angiogenic factor, leads to the assumption of an important part in angiogenesis (98). Since angiogenesis is assumed to be pivotal for endometriosis lesion

formation, the role of dendritic cells in the pathogenesis of endometriosis could be huge even if its cause or effect is not declared.

Especially a study by Izumi and colleagues is interesting. They found expanded mannose receptor positive DC in endometriosis patients, leading to increased phagocytosis and could even promote inflammation (101). These results could be of major importance in the pathogenesis of endometriosis although further confirmation is needed and maybe observing the consequences of blocking the receptor would be interesting.

3.4 Neutrophils

Neutrophil granulocytes are versatile in function but their key role in starting immune responses is important. Their lifespan compared to other immune cells is relatively short. Neutrophils descend from myeloid stem cells, their production in the bone marrow is raised by granulocyte-colony-stimulating-factor (G-CSF), which can be produced by tissue residing macrophages during inflammation processes (20,102). They leave the bone marrow completely differentiated and are the first leukocytes reaching a new inflammation side, where they are important in immediate responses and regulating the adaptive immune response (20,102). Furthermore, neutrophils can phagocytose and endocytose particles and germs leading to intracellular mortification in phagolysosomes or to antigen presenting via HLA-molecules. Due to the synthesis of cytokines and chemokines they play an important role in the regulation of the immune response. Neutrophils can store effector molecules, which are released depending on their activation (20,102).

3.4.1 Frequency

Two studies claim neutrophils to be elevated in the peritoneal fluid of women with endometriosis compared to women without the disease (36,103). Nevertheless, the number of studies about the frequency of neutrophils in endometriosis is far too slim and further studies would be required. Milewski and co-workers detected enhanced appearance of human neutrophil peptides 1, 2 and 3, which are antimicrobial peptides, α-defensins, produced by neutrophils, in the peritoneal fluid of patients with endometriosis.

Furthermore, the level of peptide increase correlated with the severity of disease, by being even higher in more severe stages (103). Interleukin 8, also known as CXCL8, was also seen to be increased in the peritoneal fluid of women with endometriosis by two study groups (103,104). This chemokine is known to attract and activate neutrophils and other immune cells to the centre of infection and is involved in angiogenesis (20).

3.4.2 Possible effect in endometriosis

To clarify the impact of neutrophils on endometriosis Takamura and co-workers performed a study with depletion of neutrophils. Therefore, they used mice, which were divided in three groups, the first group received the antibodies in the early stage, till day three, the second group in the late stage, from day eight till day twelve and the third group served as control group and did not receive any antibodies (105). In the next step they compared the number and weight of endometrial lesions in the different groups. As a result they found a significant reduce in quantity of lesions in the early stage group compared to the control group and even to the late stage group (105). Leading to the presumption of neutrophils being pivotal in the development of endometrial lesions, however further investigations need to be performed to confirm this thesis. Nevertheless, the study group found no difference in the lesion weight in the control group compared with early stage and late stage group. Only the lesion weight in the early group was diminished if compared to the late stage group (105).

Furthermore, VEGF production by neutrophils could conduct to endometriosis. An interesting study by Na and colleagues compared the VEGF production of neutrophils and monocytes, the precursor of macrophages, in endometriosis and non-endometriosis patients (106). First, they found higher levels of VEGF in the endometrial peritoneal fluid compared to the control group. Furthermore, they detected that the influence of peritoneal fluid from women with endometriosis stimulated neutrophils to produce VEGF, whereas monocytes did not produce VEGF (106). Leading to the thesis that neutrophils are responsible for the increased VEGF in endometriosis patients, and thereby possibly conduct to lesion formation and implantation. Interestingly, the study group took a look on mRNA expression of neutrophils when incubated with peritoneal fluid of women with

endometriosis in comparison to control fluids and they found an increase in mRNA expression of VEGF in the endometriosis group (106). This finding suggests that the peritoneal fluid of endometriosis patients stimulates neutrophils to produce the VEGF, which would implicate an important role of neutrophils in lesion formation. However, other studies to confirm or falsify these results could not be found and should be performed.

Furthermore, enhanced proinflammatory cytokine production by neutrophils, besides VEGF, was found. Interleukin-8 (CXCL8) and interferon-γ-induced protein 10 (CXCL10) were found to be increased in peritoneal fluid of women with endometriosis (107). The study design was set up to culture neutrophils with endometrial peritoneal fluid to detect an alteration in CXCL8 production by neutrophils. The production was increased but not alone by neutrophils but $CD4^+$ T-cells as well. The increase of CXCL10 was also caused by both cell types (107). Moreover, another cytokine produced by neutrophils localized in the endometrium could be important in endometriosis, IFN-γ. IFN-γ is a mediator that affects apoptosis, immune response and proliferation of endometrium (20,108,109). However, studies of its influence in endometriosis are missing and should be conducted.

Furthermore, another factor in conjunction with neutrophils and endometriosis are neutrophil extracellular traps (NET). If neutrophils die due to apoptosis near to centres of inflammation they pass on a net out of chromatin in the extracellular space, which is useful to catch bacteria and supports phagocytosis (20). Although it was shown that there is an increase of NETs in women with endometriosis compared to the reference group they could not verify a stimulating effect of peritoneal fluid of patients with endometriosis on neutrophil extracellular trap development (110).

Furthermore, the neutrophil-to-lymphocyte ratio (NLR), investigated in a few studies, could indicate a correlation of neutrophils and endometriosis. The NLR was measured in the blood of women with and without endometriosis, whereas studies found higher values in the endometriosis group, which also correlated with the severity of the disease (111,112). Moreover, a close connection between NLR and CA125, a tumour marker for ovarian cancer, which can be elevated in endometriosis as well, has been demonstrated (111–113). Another study in fact could detect higher NLR values for endometriosis patients with normal CA125. Thus in negative CA125 endometriosis patients NLR could be important for diagnosis (114). However, other studies found no difference in the NLR in endometriosis

and control groups (115,116). This difference in studies could result from the low specificity and sensitivity of NLR when used alone. Both specificity and sensitivity can be enhanced if measured combined with CA125 (111,112,114–116).

3.4.3 Conclusion

Current data on neutrophils in endometriosis is inconclusive. However, the few studies that exist provide similar results. The frequency of neutrophils and their products could be demonstrated to be enhanced in endometriosis. In the case of human neutrophil peptides 1, 2 and 3 the enhancement even correlated with disease severity (36,103,104). This leads to the assumption that neutrophils be involved in pathogenesis of endometriosis or at least in disease progression. However, a study with depleted neutrophils, which resulted in reduced number of lesions in the early group, indicates rather an effect in the pathogenesis. Furthermore, they found no difference in the lesion weight, which is an argument for neutrophils to be involved only in development of lesions (105). Moreover, the increased VEGF in peritoneal fluid of endometriosis patients, which is important for angiogenesis, could lead to facilitated or even be causal for implantation and lesion formation. As the study found that VEGF production is only increased in neutrophils compared to monocytes, this would point out neutrophil's role in endometriosis as well (106). Even the neutrophil-to-lymphocyte ratio, which is claimed to be able to indicate endometriosis diagnosis, could suggest an important role of neutrophils. However, the validity of NLR is controversial (111–116).

According to these study results the role of neutrophils in the pathogenesis or progression of endometriosis is highly probable. However, further studies are required to justify this suspicion.

3.5 Mast cells

Mast cells originate from myeloid progenitor cells, are durable and located in the tissue. Depending on the tissue they reside in, their lifespan adapts and their equipment of receptors and mediator production changes. The development and function of mast cells is modulated by the stem cell factor (22,102). They interact with IgE-antibodies and are

involved in immediate-type allergy. Upon activation they release their granules and what is more they survive this process. They either release preformed molecules or produce inflammatory mediators. These lead to chemotaxis and migration of other inflammatory cells. Furthermore, mast cells have a regulatory impact on the specific immune response by having an anti- or proinflammatory effect. Moreover, they affect blood circulation and permeability of vessels (22,102).

3.5.1 Mast cells in normal menstrual cycle

During the phases of the menstrual cycle mast cell numbers do not change (117–119). Solely prior to menstruation the mast cell granules were seen to lack in content, which indicates a previous activation (117). Assuming the content of mast cell granules being somehow important for menstruation. When mast cells were observed in different layers of the uterus, they were found to be present in low numbers in the endometrium (117). In the endometrium itself mast cells were located mainly in the basalis, while the myometrium contains much more mast cells, especially in the inner half of the myometrium (114,116).

During menstruation it is assumed that mast cells are involved in the shedding of the functionalis and afterwards in the regeneration of the tissue (118,121,122).

Mast cells are also discussed to be implicated in angiogenesis and so involved in cancer development (123). Likewise their increased occurrence near blood vessels contributes to that thesis as well as the observation that mast cells are involved in angiogenesis in rats during pregnancy (116,120).

Furthermore, their role in fibrosis and adhesion development is suspected (119,123), also due to their expression of receptors for fibronectin and counter receptors for vascular cell adhesion molecule-1 (VCAM-1) of the uterine mast cells (125).

3.5.2 Frequency

In the eutopic endometrium no difference was seen in frequency of mast cells during different cycle phases in women with and without the disease (126). There was also no difference in the number of mast cells in the eutopic endometrium when women with and without endometrioses were compared (127). In the ectopic lesions mast cells were seen to be increased in women with endometriosis (126–131). However, three of these studies found the increase only in comparison with eutopic endometrial tissue in women with and without endometriosis (126,127,130). Therefore, it implies that an increase in the number of mast cells could only be found in the ectopic lesions. Interestingly, an increased activation on mast cells has also been demonstrated in ectopic lesions (127,130,131), whereas the number of activated mast cells in the eutopic endometrium was very low (127,131).

3.5.3 Possible Role in Endometriosis

Activated mast cells, which are increased in lesions of endometriosis patients, are assumed to be involved in the development of fibrous adhesions. This could implicate an important role of mast cells in lesion development in endometriosis (127).

Furthermore, mast cells can produce many factors like interleukins, TNF-α, granulocyte-monocyte-stimulating factor and many more, which are able to activate macrophages, build a proinflammatory environment and act as chemotactic mediators for neutrophils and eosinophils as well. In this way they contribute to inflammatory processes by bringing immune cells into the endometrium or endometrial lesions (20,26,132).

3.5.4 Conclusion

Only a small number of studies concerning mast cells in endometriosis exist and of which only a few are useful. Due to their important role in allergies, a possible role in endometriosis does not come into consideration for many study groups because they suppose that endometriosis has no connection to allergies. Studies investigating different immune cell types do not regard mast cells intently and rank them low priority. A possible

role may be undetected due to their assumptions of not being as important as other immune cells.

Alternatively, the function of mast cells in the normal menstrual cycle could lead to the assumption of an impact in endometriosis since they are involved in inflammatory processes, which are discussed to be predominant in endometriosis patients. In addition, because of their possible role in the development of fibrous adhesions and their increased occurrence in endometrial lesions, there may be a connection to endometriosis (119,123,126–131).

However, some studies reported of a higher count of mast cells in the lesions compared to the eutopic endometrium of women with endometriosis. (126,127,130). They characterized the majority of the present mast cells within the ectopic tissue as activated and degranulated ones, which may indicate an impact of a hypersensitivity reaction and endometriosis (26,127,130–132).

3.6 Eosinophils and Basophils

Eosinophils and Basophils are subgroups of granulocytes. Eosinophiles are specialized in destroying parasites with the help of IgE-antibodies, although other pathogens can be destroyed as well. Actually, they are able to act as antigen-presenting cells too. Their granules contain enzymes, cytokines and proinflammatory mediators. Upon degranulation reactive oxygen species (ROS) are released, which are toxic and support the destruction of parasites and other pathogens (22,133,134). Maturation of eosinophils occurs in the bone marrow by the influence of different factors like IL-5, which is one of the most important ones for eosinophil maturation. Furthermore, it even supports the survival of the cells by preventing apoptosis and enhances the activation of eosinophils (133).

Basophils are known to contain histamine and also amines, proteases, proteoglycans and cytokines within their granules. Their life span is short, and they are mostly found in blood vessels, nevertheless they can also migrate into inflamed tissues. In addition, basophils produce mediators, which promote vascular permeability. Like eosinophils their maturation occurs in the bone marrow and for them interleukin-3 is their most important

mediator for maturation and differentiation (22,135). Basophils are discussed to influence the development of autoimmune diseases and allergies (135,136). Until recently, basophils were not of great scientific interest because their role in the immune system was underestimated. Only in recent years have they gained more attention, but their role in endometriosis has yet to be explored.

3.6.1 Eosinophils in normal menstrual cycle

Of all leukocytes, eosinophils make up a very small proportion (137). During luteal phase the number of eosinophils was lower than during the follicle phase. Ovulation as well led to a lower eosinophil count and a further study showed a decrease of eosinophils during menstruation (133,134). These findings lead to the assumption that eosinophils have an effect in the menstrual cycle, especially their contribution in tissue remodelling and wound healing has been discussed (138,139).

3.6.2 Eosinophils in Endometriosis

A possible effect of eosinophils in endometriosis, is currently not of scientific interest, but a few studies exist. One of these studies investigated the amount of eosinophil granulocytes in endometriosis patients. Therefore, they compared peritoneal fluid and blood samples of women with and without endometriosis. In the peritoneal fluid of endometriosis patients the eosinophil count was much higher than in control patients while no differences were found in the blood samples (140). Moreover, an early activating molecule, CD69, was observed on peripheral blood eosinophils in endometriosis patients, which was not present on the cells of the control group at all. In the peritoneal fluid it was detected in both groups, albeit the distribution was much higher in the endometriosis group (140). Another study group investigated eosinophil peroxidase, a marker for degranulated eosinophils, which was found in high intensity in samples of endometriosis patients. Interestingly, it was found nearby lesions and in the connective tissue. Apart from that eosinophil peroxidase was found only to a minor extend in control samples of the secretory phase and were completely absent in control samples of the proliferative phase

(139). Furthermore, chemoattractants and cytokines for eosinophils, like interleukin-5, eotaxin and RANTES (regulated on activator normal T-cell expressed and secreted) were investigated in woman suffering from endometriosis and healthy ones (139). The cytokine IL-5 could not be detected in the control group but enhanced levels of IL-5 were found in the endometriosis group especially in samples with high eosinophil peroxidase detection (139). Since IL-5 is important in eosinophil activation and survival the increased values of IL-5 and eosinophil peroxidase in endometriosis samples could suggest that there are more activated and viable eosinophils in endometriosis (139). However, further studies confirming these results are missing.

The chemokine RANTES was only found in low levels in endometriosis samples mainly in combination with inflammation. Otherwise eotaxin was detected in both groups, but to a greater extend in the endometriosis group (129,135). In the peritoneal fluid the amount of eotaxin was higher in women with endometriosis than in control patients. Furthermore, the same study found higher levels of eotaxin depending on the severity of the disease (141).

3.6.3 Conclusion

These results indicate a participation of eosinophils in the normal menstrual cycle. Especially, their possible involvement in menstrual shedding and wound healing could suggest a role in endometriosis as well. The enhanced occurrence of eosinophils in the peritoneal fluid of women with endometriosis supports these assumptions (140). However, the current scientific knowledge is too low to take this information for granted. Furthermore, the increased eotaxin levels in higher stages of the disease indicate rather an influence of eosinophils in the progression than in the formation of the disease (141). In fact, there are further studies needed to clarify the role of eosinophil granulocytes in the development and/or progression of endometriosis.

Since endometriosis is discussed to be an autoimmune disease and basophils are important in the formation of autoimmune diseases, basophils could be important as well in

connection with endometriosis (135,136). But other immune cells seem to be more promising candidates, which is why there are no studies on basophils and endometriosis.

3.7 B-Lymphocytes

B-Lymphocytes originate from lymphoid progenitor cells and belong to adaptive immune cells. In conjunction with T-Lymphocytes they are regarded as the cellular component of the adaptive immune system, which is known to be high in diversity and in antigen specificity. B-Lymphocytes mature in primary lymphoid organs, like the bone marrow, where they begin to express specific antigen receptors. Afterwards they migrate through the blood stream into secondary lymphoid organs, like spleen, lymph nodes and mucosa-associated lymphoid tissue (20,22,142,143). These cells are equipped with surface receptors, also known as surface immunoglobulins, for antigen recognition and they are of great diversity. If these receptors recognise and connect with the compatible antigen they are activated, and B-cells convert from naïve cells to effector cells. These effector cells, known as plasma cells, are able to produce specific antibodies for the antigen. Some of the naïve B-cells also transform into memory cells, which are important for long-term immunity and are activated by a following contact with the exact antigen (20,22,142,143).

T-helper cells are a different type of effector cells, originating from T-progenitor-cells and are able to activate antibody production by B-cells. There is a close collaboration between B- and T- Lymphocytes in immune responses (20,22,142,143). Furthermore, B-cells may contribute to autoimmune diseases and the development of allergies (142,143).

3.7.1 B-cells in menstrual cycle

The occurrence of B-cells in the endometrium of healthy women showed a peak between day six and eight of the menstrual cycle, followed by a constant low until another small increase between day 23 and 26 (144). Another study investigated B-cell and plasma cell frequency in the uterine sentinel lymph nodes in correlation with different cycle phases (145). The highest amount of these cells was found during menstruation. The number of B-cells raised between the secretory phase and menstruation and declined between menstruation and proliferative phase (145). Although the influence of the menstrual cycle

on B-cells is of interest the role of B-cells in menstrual cycle is not well investigated. But an influence of oestrogen on B-cells was found. Especially high oestrogen levels during pregnancy or maybe even preovulatory have an impact on B-cells. This effect can lead to survival of B-cells and autoreactive cells (146,147). Since highest numbers of immunoglobulins were found in the cervix preovulatory, this could indicate a high number of B-cells before ovulation and an important influence of oestrogen on the B-cell regulation (146).

3.7.2 Frequency in endometriosis

Regarding the frequency of B-cells in endometriosis different study results were found, but there is no consensus (30,144,156,157,148–155). One of these studies showed no difference of B-cells within endometrial lesions, endometrium of women with endometriosis and healthy control endometrium (148). This findings were supported by two other studies, which also did not found an alteration in B-cells within the endometrium (144,149). Similar results were found with blood samples of Gebel and co-workers, who could not show any difference in the total number of B-lymphocytes (150). Apart from that several studies present results indicating an increase in B-cells in woman suffering from endometriosis (152–157). Antsiferova for example found enhanced B-cell numbers in endometrial lesions, these B-cells even were seen to be more activated as well (152). Chishima and co-workers focused on B1-cells, a special subtype of B-lymphocytes, which are a part of the innate immune system (20,154). This subtype was elevated in peritoneal exsudate but disease severity did not correlate with B1-cell content, however they noted a non-significant trend toward higher B1-cell levels at less severe stages (154).

On the contrary two other studies found decreased B-cells in the blood (30) and in endometrial lesions (151) of endometriosis patients, although the study of Gagné and co-workers stated that the decrease was small and just statistical significant (30).

3.7.3 Role of B-Lymphocytes in endometriosis

A study by Antsiferova and co-workers investigated different B-cell types and their content. The number of B-cells in the peripheral blood did not differ in women with endometriosis but in endometriosis patients the amount of B1- and activated B-cells in endometrial lesions was higher than in the endometrium. In the lesions the numbers of these cells was increased, whereas in the eutopic endometrium of patients no difference could be found (152). Furthermore, a massive increase in mRNA IL-4 expression in lesions was detected by the same group. Although IL-4 is an interleukin, produced by T-cells and mast cells and important in B-cell activation, it could not be found in the endometrium of the patients (152).

Another study group detected upregulated genes, which are associated with immune responses, in endometriosis. They found numerous plasma cells in endometriotic lesions and upregulated mRNA of immunoglobulin G (IgG), whereas they could not detect any plasma cells in eutopic endometrium of the control group (155). Furthermore, they observed an upregulation of a protein called B-lymphocyte stimulator (BLyS), which is produced by B-cells, and it is important for the development of B-cells and differentiation of naïve B-cells into plasma cells. This protein is able to bind three different receptors. One of these receptors called BCMA was found to be upregulated in ectopic lesions as well. BCMA promotes the survival of plasma cells. BLyS was also found to be enhanced in the serum of endometriosis patients compared to the control group (155). Interestingly, this protein is increased in different autoimmune diseases as well. These findings indicate a difference in frequency and activation of B-cells in endometriosis. Especially the BLyS protein enhancement, which is normally seen in autoimmune disease, suggests an important role in endometriosis and could even demonstrate that endometriosis is an autoimmune disease (155).

The theory of autoimmune disease can be supported by another study result. Chishima and co-workers detected antinuclear antibodies in peripheral blood of some endometriosis patients (154). Antinuclear antibodies are autoantibodies, attacking body owns cell nuclei, which are elevated in autoimmune diseases (158). These antibodies were found to be correlated with increased B1-cell levels in the peritoneum (154).

Alterations in immunoglobulins could also be detected. IgG and IgA have been observed to be increased in the peritoneum in endometriosis patients. In blood samples there was just an enhancement in IgG but not in IgM levels and they noticed an enhancement in B-cells, which also correlated with the immunoglobulin levels in blood samples (153). These results implicate more active B-Lymphocytes as well. Raising the question, if endometriosis is seen as a pathogen by the immune system, leading to more activity, or if something different, leading to endometriosis, promotes this activity.

Another study found differences in IgG2 levels depending on the severity of the disease. In more severe stages of endometriosis, the amount of IgG2 was lower than in women with milder stages and the healthy control group. Interestingly, the number of B-cells did not differ in these three groups (150). These results seem to indicate reduced IgG production by B-cells in higher stages, which raises the question of what is responsible for this change in function (150). Furthermore, this study group also investigated the influence of two different drugs on IgG levels and found that treatment with danazol brought back the IgG levels in advanced endometriosis stages to the levels of the control group. Otherwise, treatment with GnRH agonists did not influence the IgG levels (150).

Since increased levels of soluble CD23, which is produced by activated B-Lymphocytes and plays a role in B-cell homeostasis, were found in the serum of patients with endometriosis, it was examined if a pharmacological treatment has an influence on CD23 production (157,159). So it was shown that soluble CD23 could be reduced in endometriosis patients when treated with danazol but not by treatment with another drug usually used for endometriosis like, leuprolide acetate, a GnRH agonist (157).

Riccio and co-workers tested another drug called Ibrutinib, which is an inhibitor of bruton-tyrosine kinase (Btk). Btk is important in maturation of B-cells and activation of mast cells (160). A deficiency in Btk for example leads to blockage of development and function of B-cells (20). In mice Ibrutinib inhibited the development of endometriosis and it reduced B-cell activation in spleen and peritoneum. This resulted in reduced volume and weight of the implants and activity was also limited. Additionally, no angiogenesis could be detected anymore (160). Ibrutinib affected not only B-cells but also macrophages, leading to increased numbers of M2 macrophages and a decrease in M1 macrophages in spleen. At

the same time, it decreased the M2 count in the peritoneum and increased M1 number there. Moreover, Ibrutinib promoted a decrease of mRNA expression for inflammatory and fibrotic mediators (160). The inhibited angiogenesis, which is important in lesion formation, through reducing B-cell function and development, implicates that B-cells are important in the angiogenesis and development of lesions. Furthermore, the diminishing effect of the drug on inflammatory and fibrotic factors, leads to the assumption that B-cells also promote inflammation and fibrogenesis and that these factors are important in endometriosis as well. These results reveal an important role of B-cells in development of endometriosis.

Programmed cell death protein-1 (PD-1) positive B-cells have been seen to be elevated in all endometriosis stages compared to control. Its ligand, PD-L1, was also increased in patients with endometriosis (156). PD-1 is an important checkpoint in the immune system, its purpose is to remain the peripheral tolerance, established by mature lymphocytes and the promotion of self-tolerance (20,156). In combination with its ligand, it is discussed to lead to T-lymphocyte activation and supports to develop inflammation and tissue injury. Furthermore, its ability to react to cancer and infections is discussed to change and even be worse in high expressing T-cells (156). Insofar, this pathway might be involved in the development of endometriosis, but further studies are therefore needed.

A gene called B-cell translocation gene 1 (BTG-1) is included in apoptosis and it prevents proliferation and adhesion (161). It is a tumour suppressor gene and its expression is not dependent on cycle phases. BTG-1 mRNA expression was seen to be reduced in lesions and endometrium of women with endometriosis compared to the healthy control group (161). The working group of Kim and co-workers hypothesized that a reduction of BTG-1 leads to decreased apoptotic functions and consequently increased cell survival, as well as enables proliferation and adhesions, which is associated with higher migration potential (161). To prove this theory, the study group transfected endometrial stromal cells, with BTG-1 siRNA for downregulation of BTG-1 expression. This downregulation results in an increased proliferation rate and an enhancement of migration and wound healing. Furthermore, a decreased number of pro-apoptotic factors and a concomitant increase in anti-apoptotic factors was detected (161). The reduced BTG-1 mRNA expression in endometriosis leads to an impairment in apoptosis and facilitated proliferation (161). Since these two factors are

discussed to be important in pathogenesis of endometriosis, these results implicate a role of BTG-1 in endometriosis. Although, the cause of the reduction is not clarified yet.

3.7.4 Anti-endometrial Antibodies

Anti-endometrial antibodies are discussed to be involved in endometriosis. These are antibodies against the endometrium, especially a reaction to endometrial glands could be found (162–164). The occurrence of these antibodies is not limited to endometriosis. In many gynaecological diseases and even in healthy patients they can be found (162,164–166). An involvement of anti-endometrial antibodies in endometriosis has not yet been clarified. The findings of different studies show controversial results. Some studies found no difference in the quantity of these antibodies compared to control groups (163,167). Otherwise, some other studies found a correlation between anti-endometrial antibodies and infertility (165,168). Alternatively, Fernandéz and co-workers detected an increased number of the antibody in the serum of women with endometriosis compared to the control group (162). A difference in correlation of disease severity (162–164,167) and menstrual cycle phases (163) could not be found. The results on frequency are not consistent due to the different methods used. Since, infertility is a problem of endometriosis patients, these antibodies could be involved, once it was found by Sarapik and co-workers that these antibodies are related to failed in vitro fertilization (166). Furthermore, it is discussed that, anti-endometrial antibodies are a consequence of the disease and not the trigger (162).

3.7.5 Conclusion

The difference in studies results regarding frequency indicate a lack of consensus across studies. Although, a trend towards increased B-lymphocytes in endometriosis patients could be seen. To confirm this hypothesis, further studies with more study participants and standardised methods should be conducted, as existing studies vary widely in terms of evaluation methods and age of studies and have low numbers of participants (150,152,153). Furthermore, a change of B-cells in activation or markers, which activate B-

cells could be found to be enhanced in endometriosis. On the one hand interleukin-4 a B-cell activator was increased and on the other hand antibodies have been elevated, which indicate higher activation of B-cells (150,152,153). Additionally, a factor, CD23, produced by activated B-cells was enhanced, which suggests increased activation of B-cells as well (157). Moreover, a possible connection of endometriosis with autoimmune diseases seems to exist. Antinuclear antibodies, which are autoantibodies, could be found in endometriosis patients and a protein called BlyS, was seen to be upregulated in B-cells, which can also be found in autoimmune diseases (154,155). Therefore, the theory of endometriosis being an autoimmune disease seems to be relevant.

Furthermore, a possible influence of B-cells in the development and progression of endometriosis can be supported by the following results. First, inhibition of a B-cell maturation factor, Btk, led to reduced development of endometriotic lesions (160). Furthermore, angiogenesis could not be detected anymore and inflammation and fibrotic markers were diminished (160). Second, another factor expressed by B-cells, PD-1^{+}, was enhanced in endometriosis, and seen to be important in inflammation and tissue injury (156). Third, a gene, BTG-1, was diminished in endometriosis, which correlates with increased proliferation, anti-apoptotic factors, migration potential and wound healing (161). These results imply an important role of B-cells in progression, proliferation, migration, survival, fibrogenesis, angiogenesis and in creating a pro-inflammatory environment, which are necessary for the pathogenesis of endometriosis. For that reason, further studies are needed to ensure the pivotal role of B-cells for the development and progression of endometriosis.

3.8 T- Lymphocytes

3.8.1 Gamma delta T-cells

Gamma delta T-cells have been detected to be important in autoimmune diseases and their progress. Therefore, they produce cytokines and chemokines, which lead to proinflammatory environments (169). In endometriosis gamma delta T-cells have not been of scientific interest, since few research has been executed. However, one study found a

difference in quantity of gamma delta T-cells in patients with endometriosis. This study group compared women with endometriosis with patients with adenomyoma and found an increase of gamma delta T-cells in the endometriosis group (170).

This could indicate that endometriosis is kind of an autoimmune disease, however, the lack of studies does not permit conclusions.

3.8.2 Natural killer-like T-cells (NKT)

Natural killer-like T-cells are a subtype of T-lymphocytes, which are similar to natural killer cells. Enabled NKT-cells generate lots of cytokines and further activate other immune cells, therefore they are important in immune reaction, especially in allergies, autoimmune processes and infections (171,172).

A study found diminished NKT cells in patients with endometriosis compared to the control group. The quantity correlated with the stage of endometriosis (172).

Another study investigated the amount of $CD56^{+}$ NK and NKT cells in patients with endometriosis and found less than in the control group (173). That could result in a decreased destruction of ectopic endometrial cells and furthermore the survival of these cells could lead to formation of endometriosis.

3.8.3 Regulatory T-cells (Treg)

Regulatory T-cells are important in the immune systems suppressive function and furthermore play a vital role in autoimmunity (174).

Gogacz and colleagues investigated the quantity of regulatory T-cells (175). They found no difference between diseased and healthy group whether in peritoneal fluid nor in peripheral blood. Further no difference could be detected regarding different menstrual cycle phases (175). Other studies could not confirm these results completely, because they detected increased Treg in peritoneal fluid in endometriosis patients compared to control (176,177), in peripheral blood no difference was found as well (176,178).

A study group found FoxP3$^+$, a Treg cell transcription factor (179), to be elevated in the secretory phase in women with the disease compared to the control group. In the menstrual and proliferative phase no differences could be found (180). Another study group could not find any differences depending on cycle phases (181,182). Interestingly, FoxP3$^+$ was found in some ectopic lesions of endometriosis patients whereas no FoxP3$^+$ could be found in the control group. The detected FoxP3$^+$ was found in the lesions and not in the surrounding tissue, which could indicate a role in the lesions itself (180).

Another study found FoxP3$^+$ to be elevated also in the eutopic endometrium in secretory phase in endometriosis (183). Although it is not clear if the FoxP3$^+$ found in the lesions is a reaction of the immune system to lesion formation or has any origin due to the disease (180). The FoxP3$^+$ cells led to impaired function of other immune cells, which would be important in detecting and removing endometrial cells. Therefore, these cells could be a support or even the trigger in the survival of endometrial tissue outside of the uterus (180). In the peritoneal fluid CD4$^+$CD25^{high} cells, another subset of Treg cells, were increased compared to control group and further the expression of FoxP3$^+$ of these cells in the peritoneal fluid was elevated in the endometriosis group too (182). Another study group could confirm the increase in CD25^{high}FoxP3$^+$ cells in peritoneal fluid of endometriosis patients though in peripheral blood a reduction was detected (184).

Chen and colleagues investigated the mRNA expression of FoxP3$^+$ in endometriosis compared to a healthy control group (185). The mRNA expression was increased in women with the diseases. Furthermore, the levels were higher in more severe stages (185). On the other hand the same study group found no difference in quantity of FoxP3$^+$ protein between endometriosis and control group (185).

Furthermore, Budiu and colleagues found increased CD4$^+$FoxP3$^+$ Tregs in mucin 1 glycoprotein (MUC1) in a mouse model of endometriosis (186). They postulate that MUC1 is enhanced in endometriosis wherefore it might be used as an endometrial lesion marker (186). This could mean that FoxP3$^+$ is involved in endometriosis and could interact with MUC1, which might have a part in endometriosis as well.

Li and colleagues regarded an interaction between a special chemokine, called thymus-expressed chemokine, or TECK, and Treg cells (187). In peritoneal fluid higher numbers of

TECK and Tregs were found to be associated with advanced stages of endometriosis. Further enhanced numbers of IL-10 and TGF-β in peritoneal fluid could be seen to be correlated with more severe stages of endometriosis (187). Another study also found enhanced TGF-β in peritoneal fluid of women with endometriosis (176). IL-10 and TGF-β are discussed to have an inhibiting function on NK-cells, especially on their cytotoxic ability (56,60). Furthermore, the study group found a diminishing effect of TECKs on apoptosis of Treg cells and on their suppressive ability (187). Further, they led to increased differentiation of regulatory T-cells and enhanced their functionality. Tregs in the following led to growth of endometriosis lesions, due to production of IL-10 and TGF-β, and Tregs were seen to contribute to proliferation and invasiveness of endometrial stromal cells (187). Therefore, the authors postulate an important role of TECKs and Tregs in endometriosis. To confirm their thesis, they injected anti-TECK in mice, which led to decreased lesion growth (187). Another study group found IL-10 and TGF-β being produced by Tregs and further anti-IL-10 and anti-TGF-β had a suppressive effect on invasion and proliferation of endometrial cells (188). This could indicate a special role of the immune system, especially of TECKs and Tregs in endometriosis through TECKs stimulating proliferation and function of Tregs and further Tregs producing cytokines leading to invasion and proliferation of endometrial tissue.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or also known as CD152 is a special protein on T-cells, especially on Tregs. This receptor inhibits and prevents an overreacting immune system. On regulatory T-cells this protein is necessary for normal functionality, which is interesting regarding Tregs importance in preventing autoimmunity (20). Liu and colleagues were interested in CTLA-4 in endometriosis (188). Therefore, they injected anti-CTLA-4 antibodies into mice. In peritoneal fluid the antibodies diminished the number of $CD4^+CD25^+$ Treg cells (188). Further Treg production of IL-10 and TGF-β was decreased and the antibody led to diminished growth and invasiveness of endometrial cells (188). These results are interesting, thus if Tregs would be leading to endometriosis through diminished prevention of autoimmunity, the experiment should have led to increased lesion formation. According to these results and the studies mentioned above a role of regulatory T-cells in pathogenesis of endometriosis cannot be excluded.

Wang and colleagues found increased CCL22 and CCL17 in a co-culture of monocytes and endometrial stromal cells combined with oestradiol and progesterone. These increased amounts had a chemotactic effect on regulatory T-cells (189). These findings could indicate an interaction of hormones and the immune system in endometriosis. Another study also detected increased amounts of CCL17 in eutopic endometrium of endometriosis patients compared to control group but they found no difference between cycle phases (190). Furthermore, these Tregs produced higher quantity of TGF-β1 as well as increased IL-1β and TNF-α, which all are proinflammatory cytokines (189). These differences in the amount of these proinflammatory cytokines could be detected in the endometrial environment. They led to increased production of IL-8 and VEGF, which are angiogenesis factors (189). Due to the importance of angiogenesis in endometriosis lesion formation this could also indicate a possible involvement in endometriosis pathogenesis. Furthermore, the co-culture led to enhanced expression of $FoxP3^+$, CTLA-4, CD73 and CD39 on Tregs, which are participating in the suppressive function of Tregs on the immune system (189).

Wei and co-workers found enhanced regulatory T-cells in the peritoneal fluid of endometriosis patients compared to control. The increase was highest in advanced stages of the disease (191). Further, they analysed a special gene called indolamine 2,3-dioxygenase (IDO1) because they found it to be more present in endometriosis than in control group. With the help of 1-Methyl-tryptophan (1-MT), an IDO-1 inhibitor, they detected an effect on Treg cells (191). The Treg cells with suppressed IDO-1 were restricted in differentiation. If 1-MT was added in vivo endometrial lesions were seen to be smaller and lighter (191). Moreover, IDO-1 was seen to be influenced by oestrogen, on the one hand IDO-1 was increased in oestrogen exposed samples and on the other hand oestrogen-receptor blocking led to downregulation (191). These findings would explain an interaction of immune cells and endometriosis, which is oestrogen dependant since oestrogen could stimulate regulatory T cell proliferation by enhancing IDO-1 (191).

3.8.4 $CD4^+$ T-cells

$CD4^+$ T-cells, known as T-helper cells, detect MHC-II-peptides, which are presented on antigen-presenting cells (20). These $CD4^+$-cells further activate the immune system and

differentiate to Th1, Th2 and Th17 cells. These cells determine if they lead to a pro- or anti-inflammatory environment. Furthermore, they support B-cells in producing antibodies (20).

The amount of $CD4^+$ cells in peritoneal fluid and peripheral blood of women with and without endometriosis was found to be not significantly different (192).

Another study detected in peritoneal fluid of patients with endometriosis $CD4^+$-T-cells to be diminished compared to control group, in peripheral blood no difference could be seen (193). This study further detected increased numbers of interleukin-10 (IL-10) especially in stage one and two of the disease compared to control (193). Interestingly, IL-10 is a cytokine, which inhibits Th1-cells and supresses release of inflammatory cytokines, whereby it is able to limit inflammatory processes (20). This could explain why ectopic endometrial cells are not destroyed by inflammation and are able to survive.

Another study detected a raised $CD4^+$ to $CD8^+$ ratio in peripheral blood of women with endometriosis in contrast to healthy women (39). In endometrium of endometriosis patients similar results could be found (148). As $CD8^+$ cells are known to be cytotoxic, this could indicate a more survival friendly environment for endometrial cells. However, another study found no difference in the ratio in peritoneal fluid when compared to the control group (194).

Th1 and Th2 $CD4^+$ cells

Th1 cells are important in infections by microorganisms, especially in detecting infected macrophages (20). Th2 cells are relevant in infections by parasites. Further, they play an important role in IgE-production by B-cells. IgE-antibodies are responsible for asthma and allergies, which is interesting regarding endometriosis and its possible cause (20).

Th1 and Th2 $CD4^+$ cells act just like antagonists, by inhibiting each other through their secreted cytokines. Whereas Th1 cells lead to pro-inflammatory reactions, Th2 cause a more anti-inflammatory response (26,195). Further, Th1 cells promote cell-mediated immunity by activating $CD8^+$ differentiation, monocytes and macrophages and by producing different cytokines, like TNF-α, IFN-γ, IL-2 and IL-12 (196). Previous studies even found a connection to pregnancy. Th2 was seen to be connected with fetal survival,

whereas Th1 was associated with infertility or even loss of pregnancy (109,197), which could indicate a role in implantation. Since Th2 $CD4^+$ cells evoke an anti-inflammatory process, a possible role in endometriosis, by inhibiting a required reaction against endometrial cells, could be assumed.

The number of Th1 cells have been seen to be decreased in lesions compared to eutopic endometrium (178). However, in peripheral blood of women with endometriosis Th1 cells were increased compared to the healthy control group, on the other hand no difference was found in Th2 cells (178). Furthermore, Th1 was seen to be associated with severity of the disease, especially proinflammatory cytokines secreted by Th1 cells, were increased in peritoneal fluid in endometriosis. This increase particularly was related to more severe stages (195). However, another study could not find differences in Th1 in peritoneal fluid but Th2 was increased in peritoneal fluid of women with endometriosis (177).

These findings could indicate a role of Th1 $CD4^+$ cells in endometriosis by inducing a pro-inflammatory environment, however, the current information is insufficient to make conclusions.

The two subtypes of $CD4^+$ cells, Th1 and Th2, especially their development in endometriosis compared with a control group was of interest for the study group of Chen (198). GATA-3 and T-bet are transcription factors for Th1 and Th2 T-cells. T-bet stimulates Th1 and inhibits Th2 development whereas GATA-3 acts contrary (20,198). In endometriosis GATA-3 mRNA expression was increased, T-bet expression was similar to the control group and in endometrial tissue GATA-3 protein was enhanced and T-bet protein was diminished (198). These findings could explain an increase of Th2 cells.

Furthermore, in eutopic endometrium mRNA of IL-10, which is typical for Th2 cells, was seen to be higher in patients witch endometriosis compared to mRNA of interleukin-2 (IL-2), typical for Th1 cells (152). This could indicate that Th2 cells are predominant in endometriosis. In peripheral blood mRNA levels of IL-10 and interleukin-4 (IL-4) was seen to be increased, IL-2 was diminished in endometriosis group compared to control (152). IL-4 mRNA, which is typical for Th2, could not be detected in eutopic samples. In ectopic endometrium IL-4 was increased and IL-10 was decreased (152). IL-4 activates B-cells (20)

and further is discussed to lead to fibrosis (199), which could be interesting regarding endometriosis.

$CD4^+$ Th17 cells

Th17 cells are involved in reactions against extracellular bacteria and fungi. Furthermore, they secrete IL-17 and IL-22, which are important in epithelia of genitourinary, respiratory and intestinal tract, where they prevent infiltration by microorganisms (20).

$CD4^+$ Th17 cells in patients with endometriosis were increased in peritoneal fluid and peripheral blood compared to control group (26,200). Furthermore, in patients with advanced endometriosis Th17 levels in peritoneal fluid were higher than in mild stages, in peripheral blood no difference was found (200).

Another study detected higher numbers of Th17 cells in endometrial lesions compared to endometrium but could not find a difference in peripheral blood comparing patients with and without endometriosis (178) as it was also shown by Gogacz and co-workers (200). In peritoneal fluid of women with and without endometriosis the number of Th17 cells was similar (177).

Interleukin-17A (IL-17A), a product of Th17 cells and other immune cells, is of interest because it leads to proliferation of endometrial stromal cells (201), which could be important in endometriosis. Zhang and colleagues found IL-17 in peritoneal fluid of women with less severe endometriosis being increased in comparison to advanced endometriosis and healthy women (202). That could indicate a role of IL-17 and therefore maybe Th17 in early endometriosis, especially in proliferation of lesions. However, another study could not detect any differences of IL-17A in peritoneal fluid and peripheral blood between endometriosis and control group (203).

3.8.5 $CD8^+$ T-cells

$CD8^+$ T-cells also known as cytotoxic T-cells, recognize MHC-I peptides of infected cells and eliminate them (20).

The amount of $CD8^+$ cells in endometrium of women with and without endometriosis was seen to be similar throughout the cycle. Further, $CD8^+$ cells were not reduced as much as $CD4^+$ cells, after proliferative phase. The $CD4^+$ to $CD8^+$ ratio, was similar in proliferative phase, while $CD8^+$ became more dominant, towards the cycle end (144).

Although, in peripheral blood no difference of $CD8^+$ cells throughout the cycle in women with endometriosis was detected there was a change of $CD8^+$ cells in healthy women (204). On the contrary, in ectopic endometrial lesions $CD8^+$ cells were more abundant than in eutopic endometrium of the control group (148).

The lack of different studies and information does not allow conclusions. Further, studies should be regarded.

4. Discussion and Conclusion

Endometriosis is a disease affecting many women worldwide. Despite the high incidence of endometriosis, little is known of its origin. Since changes of the immune cells in endometriosis have been observed, their role in the emergence and formation of the disease has been under discussion.

The number of studies to different immune cell types vary strongly. One reason for this could be, that the conductors of studies choose immune cells of interest based on their normal function and as a consequence their presumable effect in endometriosis. On the one hand for example some cells are known for their involvement in allergies and as no connection between allergies and endometriosis is assumed, these cells are not of studies interest. On the other hand, different types of immune cells, which would have a plausible role in endometriosis, are studied in more detail. Maybe this is a possible target for mistakes in the scientific research for the origin of endometriosis, by selecting the unlikely out. Furthermore, different results, especially in terms of the quantity of different immune cells in endometriosis patients, are not allowing to directly connect a certain type of immune cells to endometriosis.

Angiogenesis is seen as an important step in endometriosis evolution. Macrophages were seen to have an impact on vessel formation (73,86–88). A study of Bacci and co-workers even connected inhibited macrophages with diminished vessel and lesion formation (78). A special type of macrophages ($Tie2^{+}$), which are important in angiogenesis have been found in endometriosis. Selectively inhibiting these cells led to decreased lesion growth and loss of vessel formation (89). Furthermore, macrophages can produce VEGF, which is important in angiogenesis and vessel formation (71,73). In studies with mice, lacking of VEGF receptors, fewer vessels were detected (90).

Dendritic cells were seen to infiltrate lesions and have VEGF receptors. Further, dendritic cells have been seen to be next to new blood vessel, which could indicate a role in formation of lesions (98). However, implanting dendritic cells into lesions in mice, promoted lesion growth though no difference in vessel formation was seen (98).

Furthermore, VEGF was seen to be increased in peritoneal fluid of patients with endometriosis. Both macrophages and neutrophils are known to produce VEGF. Thus, a

study compared VEGF production of neutrophils and monocytes, which are precursors of macrophages, under the influence of peritoneal fluid of endometriosis patients. An increase in VEGF formation was found in neutrophils, whereas there was no synthesis found in monocytes. Therefore, a possible role of neutrophils in vessel formation and further implantation can be assumed, especially as peritoneal fluid of endometriosis stimulated VEGF production (106). However, it is not clear if this is a step of endometriosis evolution or origin and further there was just one study finding these results. Further studies should be done to confirm these results. But if these results could be approved, inhibition of angiogenesis in lesions could be a possible target for therapy.

Mast cells have been found next to blood vessel as well and are discussed to be involved in angiogenesis in pregnant rats (119,120,123–125). Although the role of mast cells in angiogenesis in endometriosis has not been studied yet.

Inhibiting a maturation factor of B-cells resulted in inhibition of endometriosis and further no angiogenesis could be detected anymore, which could indicate a role of B-cells in angiogenesis as well (160).

Products of regulatory T-cells were seen to lead to increase of VEGF, which could also indicate an involvement in angiogenesis of endometriosis (189).

To conclude, angiogenesis is important in endometriosis and many different immune cell types influence angiogenesis presumably. Especially, neutrophils seem to be of interest concerning angiogenesis, which needs to be studied in more detail.

Endometriosis is also discussed to have similarities to autoimmune diseases or even that it is an autoimmune disease. Although basophiles are discussed to contribute to autoimmune diseases, their role in endometriosis has not been of great scientific interest (135,136). T-cells are also seen to be involved in autoimmune diseases. For example gamma-delta T-cells, which have been seen to be increased in endometriosis but there are just a few studies to gamma-delta T-cells in endometriosis, which is why their role in endometriosis is not clear yet (169,170). Natural killer-like T-cells and regulatory T-cells have also been involved in autoimmunity (171,172,174). B-cells are also discussed to be important in autoimmune diseases (142,143). A protein produced by B-cells, which is increased in different autoimmune diseases, was also elevated in endometriosis (155). This protein

leads to differentiation of B-cells because of that a difference in activation and quantity of B-cells in endometriosis can be assumed (155). Another factor, antinuclear antibodies, which are also typical in autoimmune diseases were also seen to be increased in endometriosis (154,158). Further, higher amounts of antinuclear antibodies have been linked to increased B-cells in the peritoneum of endometriosis patients (154,158). These findings suggest a possible connection between endometriosis and autoimmune diseases. Therefore, a possible target for therapy may be autoimmune therapy, but further studies should be performed.

Concerning the quantity of different immune cell types in endometriosis, it is difficult to find a conclusion. For some cell types only a few or even just one study exists regarding their amount, while for other immune cell types there are a lot of studies. The problem is that one study is insufficient to draw a conclusion and in the other case the results of the studies often vary greatly and even contradict each other. The quantity changes of different cell types would be of interest. Due to their functions a role in endometriosis could be assumed and a priorisation in the involvement of the immune cell types could be realized and further studies could focus on a few cell types. However, as mentioned above it is difficult to conclude. Besides the frequency of immune cells, their functionality in endometriosis is also of interest. Many studies found changes in the effect of immune cells in endometriosis. The cytotoxic ability of natural killer cells has been seen to be altered in endometriosis. However, some studies found diminished activity (28,35,42–44) whereas others found no difference (44,45). A diminished cytotoxic activity of NK-cells could explain a survival of endometrial cells in endometriosis. One study even found diminished cytotoxic ability against endometrial cells (27). Furthermore, phagocytic ability of macrophages was seen to be reduced in endometriosis (82–84), which could also explain a survival of endometrial cells. A study even found macrophages of diseased women leading to improved proliferation of endometrial cells, whereas macrophages of healthy women inhibited proliferation (85). Activated mast cells, have been detected to be increased in ectopic (127,130,131) and decreased in eutopic lesions (127,131). As a result, an important role of mast cells in lesion development can be assumed. B-lymphocyte activators and antibodies have been seen to be increased and a factor produced by activated B-cells was also enhanced in diseased women (150,152,153,157). This indicates that B-lymphocytes

are more activated in endometriosis and may be important in inflammation processes in endometriosis.

Factors inhibiting NK-cells, especially their cytotoxic ability and further Tregs suppressive function, have been found to be increased (56,60,187). By inhibiting these factors, proliferation and invasion of endometrial cells in endometriosis has been reduced as well (188). Leading to the assumption that regulatory T-cells and their variations are important in endometriosis.

There are many studies to endometriosis however, some areas of interest are lacking of studies. The current knowledge implies that immune cells are important in endometriosis. Although it remains unclear if their role is just in the further development of the disease after the emergence or if they are important in the origin itself. Based on the results outlined in this work, a special role in lesion formation and development in endometriosis can be supposed and further seen as a possible target for therapy. Especially a few studies, which investigated inhibition or activation of immune cell types in endometriosis leading to changes of lesions, showed that it should be investigated further and has great potential (105,157,160,187,188,205). Even if it would not be a curing therapy, the reduction of lesions concomitant with a possible reduction of the massive pain patients are suffering of, would be a big step forward for women with endometriosis.

Literaturverzeichnis

1. Debus G, Schuhmacher I. Gynäkologie und Geburtshilfe [Internet]. 4. vollstä. Weyerstahl T, Stauber M, editors. Stuttgart: Georg Thieme Verlag; 2013. 297–303 p. Available from: https://eref.thieme.de/10.1055/b-002-94109

2. Kaufmann M, Costa SD, Scharl A. Die Gynäkologie. 3. vollstä. Berlin Heidelberg: Springer Verlag; 2013. 287–305 p.

3. Koujyo T, Hatakeyama S, Yamada H, Iwabuchi K, Kajino K, Ogasawara K, et al. Induction of endometriosis and adenomyosis by transvaginal pituitary transplantation in mice with and without natural killer cell activity. Am J Reprod Immunol. 1998;40(6):441–6.

4. Thiruchelvam U, Wingfield M, O'Farrelly C. Natural Killer Cells: Key Players in Endometriosis. Am J Reprod Immunol [Internet]. 2015 Oct [cited 2019 May 24];74(4):291–301. Available from: http://doi.wiley.com/10.1111/aji.12408

5. LIU DTY, HITCHCOCK A. Endometriosis: its association with retrograde menstruation, dysmenorrhoea and tubal pathology. BJOG An Int J Obstet Gynaecol. 1986;93(8):859–62.

6. Sampson JA. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. Am J Pathol. 1927 Mar;3(2):93-110.43.

7. Troncon J, Zani A, Vieira A, Poli-Neto O, Nogueira A, Rosa-E-Silva J. Endometriosis in a patient with Mayer-Rokitansky-Küister-Hauser syndrome. Gazz Medica Ital Arch per le Sci Mediche. 2014;173(3):141–3.

8. Mok-Lin EY, Wolfberg A, Hollinquist H, Laufer MR. Endometriosis in a Patient with Mayer-Rokitansky-Küster-Hauser Syndrome and Complete Uterine Agenesis: Evidence to Support the Theory of Coelomic Metaplasia. J Pediatr Adolesc Gynecol [Internet]. 2010 Feb;23(1):e35–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083318809001296

9. Martin JD, Hauck AE. Endometriosis in the male. Am Surg. 1985 Jul;51(7):426–30.

10. Agarwal SK, Chapron C, Giudice LC, Al. E. Clinical diagnosis of endometriosis : a call to action. Am J Obstet Gynecol [Internet]. 2019;220(4):354.e1-354.e12. Available from: https://doi.org/10.1016/j.ajog.2018.12.039

11. Silbernagl S, Lang F. Taschenatlas Pathophysiologie. 5. unverän. Stuttgart: Thieme; 2018. 302–305 p.

12. Casper RF. Progestin-only pills may be a better fi rst-line treatment for endometriosis than combined estrogen-progestin contraceptive pills. Fertil Steril [Internet]. 2017;1–4. Available from: http://dx.doi.org/10.1016/j.fertnstert.2017.01.003

13. Harada T, Momoeda M, Taketani Y, Hoshiai H. Low-dose oral contraceptive pill for dysmenorrhea associated with endometriosis : a placebo-controlled , double-blind , randomized trial. Fertil Steril [Internet]. 2008;90(5):1583–8. Available from: http://dx.doi.org/10.1016/j.fertnstert.2007.08.051

14. Harada T, Momoeda M, Terakawa N, Taketani Y. Evaluation of a low-dose oral contraceptive pill for primary dysmenorrhea : a placebo-controlled , double-blind , randomized trial. Fertil Steril [Internet]. 2011;95(6):1928–31. Available from: http://dx.doi.org/10.1016/j.fertnstert.2011.02.045

15. Falcone T, Flyckt R. Clinical Management of Endometriosis. Obstet Gynecol [Internet]. 2018

Mar;131(3):557–71. Available from: https://journals.lww.com/00006250-201803000-00023

16. Georgiou EX, Melo P, Baker PE, Sallam HN, Arici A, Garcia-Velasco JA, et al. Long-term GnRH agonist therapy before in vitro fertilisation (IVF) for improving fertility outcomes in women with endometriosis. Cochrane Database Syst Rev [Internet]. 2019 Nov 20 [cited 2020 Feb 17];(11). Available from: http://doi.wiley.com/10.1002/14651858.CD013240.pub2

17. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis : pathogenesis and treatment. Nat Publ Gr [Internet]. 2013;10(5):261–75. Available from: http://dx.doi.org/10.1038/nrendo.2013.255

18. Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Viganò P, Fedele L. The effect of surgery for symptomatic endometriosis: the other side of the story. Hum Reprod Update [Internet]. 2009 Jan 9;15(2):177–88. Available from: https://doi.org/10.1093/humupd/dmn062

19. Silbernagl S, Despopoulos A. Taschenatlas Physiologie. 8. Auflage. Vol. 68. Stuttgart: Thieme; 2012.

20. Murphy K, Weaver C. Janeway Immunologie. 9. Auflage. Janeway Immunologie. Berlin: Springer Spektrum; 2018.

21. Montel-Hagen A, Crooks GM. From pluripotent stem cells to T cells. Exp Hematol. 2019;71:24–31.

22. Saloga J, Klimex L, Buhl R, Mann W, Knop J, Grabbe S. Allergologie-Handbuch: Grundlagen und klinische Praxis. 2.Auflage. Stuttgart: Schattauer; 2012.

23. Kumar V. A STING to inflammation and autoimmunity. J Leukoc Biol. 2019;106(1):171–85.

24. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. 2012;249:1–18. Available from: papers://1825b63a-f617-4840-9a49-fa78b8732348/Paper/p1178

25. Ścieżyńska, Komorowski, Soszyńska, Malejczyk. NK Cells as Potential Targets for Immunotherapy in Endometriosis. J Clin Med. 2019;8(9):1468.

26. Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update. 2019 Sep;25(5):564–91.

27. Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. Fertil Steril [Internet]. 1991;56(1):45–51. Available from: http://dx.doi.org/10.1016/S0015-0282(16)54414-8

28. Garzetti GG, Ciavattini A, Provinciali M, Muzzioli M, Stefano G Di, Fabris N. Natural killer activity in stage III and IV endometriosis: Impaired cytotoxicity and retained lymphokine responsiveness of natural killer cells. Gynecol Endocrinol. 1995;9(2):125–30.

29. Maeda N, Izumiya C, Kusum T, Masumoto T, Yamashita C, Yamamoto Y, et al. Killer Inhibitory Receptor CD158a Overexpression Among Natural Killer Cells in Women With Endometriosis is Undiminished by Laparoscopic Surgery and Gonadotropin Releasing Hormone Agonist Treatment*. Am J Reprod Immunol. 2004;51(5):364–72.

30. Gagné D, Rivard M, Pagé M, Shazand K, Hugo P, Gosselin D. Blood leukocyte subsets are modulated in patients with endometriosis. Fertil Steril. 2003;80(1):43–53.

31. Matsuoka S, Maeda N, Izumiya C, Yamashita C, Nishimori Y, Fukaya T. Expression of

Inhibitory-Motif Killer Immunoglobulin-Like Receptor, KIR2DL1, is Increased in Natural Killer Cells from Women with Pelvic Endometriosis. Am J Reprod Immunol. 2005;53(5):249–54.

32. Maeda N, Izumiya C, Oguri H, Kusume T, Yamamoto Y, Fukaya T. Aberrant expression of intercellular adhesion molecule-1 and killer inhibitory receptors induces immune tolerance in women with pelvic endometriosis. Fertil Steril. 2002;77(4):679–83.

33. Galandrini R, Ph D, Porpora G, Stoppacciaro A. Increased frequency of human leukocyte antigen – E inhibitory receptor CD94 / NKG2A – expressing peritoneal natural killer cells in patients with endometriosis. 2008;89(May).

34. Zhang C, Maeda N, Izumiya C, Yamamoto Y, Kusume T, Oguri H, et al. Killer immunoglobulin-like receptor and human leukocyte antigen expression as immunodiagnostic parameters for pelvic endometriosis. Am J Reprod Immunol. 2006;55(2):106–14.

35. Oosterlynck DJ, Lacquet FA, Waer M, Koninckx PR. Lymphokine-activated killer activity in women with endometriosis. Gynecol Obstet Invest. 1994;37(3):185–90.

36. Tariverdian N, Siedentopf F, Rücke M, Blois SM, Klapp BF, Kentenich H, et al. Intraperitoneal immune cell status in infertile women with and without endometriosis. J Reprod Immunol. 2009;80(1–2):80–90.

37. Kang YJ, Jeung IC, Park A, Park YJ, Jung H, Kim TD, et al. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Hum Reprod. 2014;29(10):2176–89.

38. Dias JA, Podgaec S, de Oliveira RM, Carnevale Marin ML, Baracat EC, Abrão MS. Patients With Endometriosis of the Rectosigmoid Have a Higher Percentage of Natural Killer Cells in Peripheral Blood. J Minim Invasive Gynecol. 2012;19(3):317–24.

39. Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-bartosinska A. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediators Inflamm [Internet]. 2003 Jun [cited 2019 May 24];12(3):131–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12857596

40. Giuliani E, Parkin KL, Lessey BA, Young SL, Fazleabas AT. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. Am J Reprod Immunol. 2014 Sep;72(3):262–9.

41. Thiruchelvam U, Wingfield M, O'Farrelly C. Increased uNK Progenitor Cells in Women With Endometriosis and Infertility are Associated With Low Levels of Endometrial Stem Cell Factor. Am J Reprod Immunol. 2016;75(4):493–502.

42. Oosterlynck DJ, Meuleman C, Waer M, Vandeputte M, Koninckx PR. The natural killer activity of peritoneal fluid lymphocytes is decreased in women with endometriosis. Fertil Steril [Internet]. 1992;58(2):290–5. Available from: http://dx.doi.org/10.1016/S0015-0282(16)55224-8

43. Wu MY, Yang JH, Chao KH, Hwang JL, Yang YS, Ho HN. Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis. Fertil Steril. 2000;74(6):1187–91.

44. Ho HN, Chao KH, Chen HF, Wu MY, Yang YS, Lee TY. Peritoneal natural killer cytotoxicity and CD25+CD3+ lymphocyte subpopulation are decreased in women with stage III-IV endometriosis. Hum Reprod. 1995;10(10):2671–5.

45. Vigano P, Vercellini P, Di Blasio AM, Colombo A, Candiani GB, Vignali M. Deficient

antiendometrium lymphocyte-mediated cytotoxicity in patients with endometriosis. Fertil Steril. 1991;56(5):894–9.

46. Jondal M. The human NK cell--a short over-view and an hypothesis on NK recognition. Clin Exp Immunol [Internet]. 1987 Nov;70(2):255–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2892600

47. Freud AG, Mundy-Bosse BL, Yu J, Caligiuri MA. The broad spectrum of human natural killer cell diversity. Immunity. 2017;176:100–106.

48. Di Vito C, Mikulak J, Mavilio D. On the way to become a natural killer cell. Front Immunol. 2019;10(August):1–15.

49. Faas MM, de Vos P. Uterine NK cells and macrophages in pregnancy. Placenta [Internet]. 2017;56:44–52. Available from: http://dx.doi.org/10.1016/j.placenta.2017.03.001

50. Bulmer JN, Lash GE. Uterine natural killer cells: Time for a re-appraisal? F1000Research. 2019;8:1–11.

51. Maeda N, Izumiya C, Yamamoto Y, Oguri H, Kusume T, Fukaya T. Increased killer inhibitory receptor KIR2DL1 expression among natural killer cells in women with pelvic endometriosis. Fertil Steril. 2002;77(2):297–302.

52. Kawashima M, Maeda N, Adachi Y, Takeuchi T, Yamamoto Y, Izumiya C, et al. Human leukocyte antigen-G, a ligand for the natural killer receptor KIR2DL4, is expressed by eutopic endometrium only in the menstrual phase. Fertil Steril. 2009;91(2):343–9.

53. Del Mar Vernet-Tomás M, Pérez-Ares CT, Verdú N, Molinero JL, Fernández-Figueras MT, Carreras R. The endometria of patients with endometriosis show higher expression of class I human leukocyte antigen than the endometria of healthy women. Fertil Steril. 2006;85(1):78–83.

54. De Placido G, Alviggi C, Di Palma, Carravetta C, Matarese G, Landino G, et al. Serum concentrations of soluble human leukocyte class I antigens and of the soluble intercellular adhesion molecule-1 in endometriosis: Relationship with stage and non-pigmented peritoneal lesions. Hum Reprod. 1998;13(11):3206–10.

55. Matalliotakis IM, Athanassakis I, Goumenou AG, Neonaki MA, Koumantakis EE, Vassiliadis S, et al. The possible anti-inflammatory role of circulating human leukocyte antigen levels in women with endometriosis after treatment with danazol and leuprorelin acetate depot. Mediators Inflamm. 2001;10(2):75–80.

56. Guo SW, Du Y, Liu X. Platelet-derived TGF-βI mediates the down-modulation of NKG2D expression and may be responsible for impaired natural killer (NK) cytotoxicity in women with endometriosis. Hum Reprod. 2016;31(7):1462–74.

57. González-Foruria I, Santulli P, Chouzenoux S, Carmona F, Batteux F, Chapron C. Soluble ligands for the NKG2D receptor are released during endometriosis and correlate with disease severity. PLoS One. 2015;10(3):1–15.

58. Sikora J, Smycz-Kubańska M, Mielczarek-Palacz A, Bednarek I, Kondera-Anasz Z. The involvement of multifunctional TGF-β and related cytokines in pathogenesis of endometriosis. Immunol Lett [Internet]. 2018;201:31–7. Available from: https://doi.org/10.1016/j.imlet.2018.10.011

59. Yu JJ, Sun HT, Zhang ZF, Shi RX, Liu LB, Shang WQ, et al. IL15 promotes growth and invasion of endometrial stromal cells and inhibits killing activity of NK cells in endometriosis.

Reproduction. 2016;152(2):151–60.

60. Zhou W-J, Yang H-L, Shao J, Mei J, Chang K-K, Zhu R, et al. Anti-inflammatory cytokines in endometriosis. Cell Mol Life Sci [Internet]. 2019 Jun 2 [cited 2019 May 26];76(11):2111–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30826860

61. Wu MY, Ho HN. The role of cytokines in endometriosis. Am J Reprod Immunol. 2003;49(5):285–96.

62. Funamizu A, Fukui A, Kamoi M, Fuchinoue K, Yokota M, Fukuhara R, et al. Expression of Natural Cytotoxicity Receptors on Peritoneal Fluid Natural Killer Cell and Cytokine Production by Peritoneal Fluid Natural Killer Cell in Women with Endometriosis. Am J Reprod Immunol. 2014;71(4):359–67.

63. Hassa H, Tanir HM, Tekin B, Kirilmaz SD, Sahin Mutlu F. Cytokine and immune cell levels in peritoneal fluid and peripheral blood of women with early- and late-staged endometriosis. Arch Gynecol Obstet. 2009;279(6):891–5.

64. Mazzeo D, Viganó P, Di Blasio AM, Sinigaglia F, Vignali M, Panina-Bordignon P. Interleukin-12 and its free p40 subunit regulate immune recognition of endometrial cells: Potential role in endometriosis. J Clin Endocrinol Metab. 1998;83(3):911–6.

65. Nishida M, Nasu K, Ueda T, Fukuda J, Takai N, Miyakawa I. Endometriotic cells are resistant to interferon-γ-induced cell growth inhibition and apoptosis: A possible mechanism involved in the pathogenesis of endometriosis. Mol Hum Reprod. 2005;11(1):29–34.

66. Ho HN, Wu MY, Chao KH, Chen CD, Chen SU, Chen HF, et al. Decrease in interferon gamma production and impairment of T-lymphocyte proliferation in peritoneal fluid of women with endometriosis. Am J Obstet Gynecol. 1996;175(5):1236–41.

67. Barcz E, Milewski Ł, Dziunycz P, Kamiński P, Płoski R, Malejczyk J. Peritoneal cytokines and adhesion formation in endometriosis: An inverse association with vascular endothelial growth factor concentration. Fertil Steril. 2012;97(6).

68. Izumi G, Koga K, Takamura M, Makabe T, Satake E, Takeuchi A, et al. Involvement of immune cells in the pathogenesis of endometriosis. J Obstet Gynaecol Res [Internet]. 2018 Feb [cited 2019 May 13];44(2):191–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29316073

69. Jensen AL, Collins J, Shipman EP, Wira CR, Guyre PM, Pioli PA. A subset of human uterine endometrial macrophages is alternatively activated. 2012;23:1–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

70. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677–86.

71. Ning F, Liu H, Lash GE. The Role of Decidual Macrophages During Normal and Pathological Pregnancy. Am J Reprod Immunol. 2016;75(3):298–309.

72. Gautier. Gene expression profiles and transcriptional regulatory pathways underlying mouse tissue macrophage identity and diversityGautier. 2013. “Gene Expression Profiles and Transcriptional Regulatory Pathways Underlying Mouse Tissue Macrophage Identity and Dive. Nat Immunol. 2013;13(11):1118–28.

73. Hogg C, Horne AW, Greaves E. Endometriosis-Associated Macrophages: Origin, Phenotype, and Function. Front Endocrinol (Lausanne) [Internet]. 2020 Jan 23;11(January):1–15.

Available from: https://www.frontiersin.org/article/10.3389/fendo.2020.00007/full

74. Hill JA, Faris HMP, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril [Internet]. 1988;50(2):216–22. Available from: http://dx.doi.org/10.1016/S0015-0282(16)60062-6

75. Berbic M, Schulke L, Markham R, Tokushige N, Russell P, Fraser IS. Macrophage expression in endometrium of women with and without endometriosis. Hum Reprod. 2009;24(2):325–32.

76. Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Ishimaru T. Differential macrophage infiltration in early and advanced endometriosis and adjacent peritoneum. Fertil Steril. 2004;81(3):652–61.

77. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. Subpopulations of Macrophages within Eutopic Endometrium of Endometriosis Patients. Am J Reprod Immunol. 2015;73(3):221–31.

78. Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol [Internet]. 2009;175(2):547–56. Available from: http://dx.doi.org/10.2353/ajpath.2009.081011

79. Cominelli A, Gaide Chevronnay HP, Lemoine P, Courtoy PJ, Marbaix E, Henriet P. Matrix metalloproteinase-27 is expressed in CD163+/CD206+M2 macrophages in the cycling human endometrium and in superficial endometriotic lesions. Mol Hum Reprod. 2014;20(8):767–75.

80. Bain CC, Jenkins SJ. The biology of serous cavity macrophages. Cell Immunol [Internet]. 2018;330(November 2017):126–35. Available from: https://doi.org/10.1016/j.cellimm.2018.01.003

81. Jenkins SJ, Ruckerl D, Cook PC, Jones LH, Fred D, Rooijen N Van, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of Th2 inflammation *. 2011;332(6035):1284–8.

82. Wu MH, Shoji Y, Wu MC, Chuang PC, Lin CC, Huang MF, et al. Suppression of matrix metalloproteinase-9 by prostaglandin E2 in peritoneal macrophage is associated with severity of endometriosis. Am J Pathol [Internet]. 2005;167(4):1061–9. Available from: http://dx.doi.org/10.1016/S0002-9440(10)61195-9

83. Chuang PC, Lin YJ, Wu MH, Wing LYC, Shoji Y, Tsai SJ. Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. Am J Pathol [Internet]. 2010;176(2):850–60. Available from: http://dx.doi.org/10.2353/ajpath.2010.090551

84. Chuang P-C, Wu M-H, Shoji Y, Tsai S-J. Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. J Pathol. 2009;(June):231–41.

85. Braun DP, Muriana A, Gebel H, Rotman C, Rana N, Dmowski WP. Monocyte-mediated enhancement of endometrial cell proliferation in women with endometriosis. Fertil Steril [Internet]. 1994;61(1):78–84. Available from: http://dx.doi.org/10.1016/S0015-0282(16)56456-5

86. Garry R, Hart R, Karthigasu KA, Burke C. Structural changes in endometrial basal glands during menstruation. BJOG An Int J Obstet Gynaecol. 2010;117(10):1175–85.

87. Sharkey AM, Day K, McPherson A, Malik S, Licence D, Smith SK, et al. Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J Clin Endocrinol Metab. 2000;85(1):402–9.

88. Eidukaite A, Tamosiunas V. Endometrial and peritoneal macrophages: Expression of activation and adhesion molecules. Am J Reprod Immunol. 2004;52(2):113–7.

89. Capobianco A, Monno A, Cottone L, Venneri MA, Biziato D, Di Puppo F, et al. Proangiogenic Tie2+ macrophages infiltrate human and murine endometriotic lesions and dictate their growth in a mouse model of the disease. Am J Pathol [Internet]. 2011;179(5):2651–9. Available from: http://dx.doi.org/10.1016/j.ajpath.2011.07.029

90. Sekiguchi K, Ito Y, Hattori K, Inoue T, Hosono K, Honda M, et al. VEGF Receptor 1-Expressing Macrophages Recruited from Bone Marrow Enhances Angiogenesis in Endometrial Tissues. Sci Rep. 2019;9(1):1–14.

91. Chan RWS, Lee CL, Ng EHY, Yeung WSB. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. Cell Prolif. 2017;50(3):1–9.

92. Shao J, Zhang B, Yu JJ, Wei CY, Zhou WJ, Chang KK, et al. Macrophages promote the growth and invasion of endometrial stromal cells by downregulating IL-24 in endometriosis. Reproduction. 2016;152(6):673–82.

93. Mei J, Chang KK, Sun HX. Immunosuppressive macrophages induced by IDO1 promote the growth of endometrial stromal cells in endometriosis. Mol Med Rep. 2017;15(4):2255–60.

94. Abomaray F, Gidlöf S, Götherström C. Mesenchymal Stromal Cells Are More Immunosuppressive *In Vitro* If They Are Derived from Endometriotic Lesions than from Eutopic Endometrium. Stem Cells Int [Internet]. 2017;2017:1–13. Available from: https://www.hindawi.com/journals/sci/2017/3215962/

95. Schulke L, Manconi F, Markham R, Fraser IS. Endometrial dendritic cell populations during the normal menstrual cycle. Hum Reprod. 2008;23(7):1574–80.

96. Schulke L, Berbic M, Manconi F, Tokushige N, Markham R, Fraser IS. Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. Hum Reprod. 2009;24(7):1695–703.

97. Maridas DE, Hey-Cunningham AJ, Ng CHM, Markham R. Peripheral and endometrial dendritic cell populations during the normal cycle and in the presence of endometriosis. Physiol Behav. 2014;176(12):139–48.

98. Fainaru O, Adini A, Benny O, Adini I, Short S, Bazinet L, et al. Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. FASEB J. 2008;22(2):522–9.

99. Pencovich N, Luk J, Hantisteanu S, Hornstein MD, Fainaru O. The development of endometriosis in a murine model is dependent on the presence of dendritic cells. Reprod Biomed Online [Internet]. 2014;28(4):515–21. Available from: http://dx.doi.org/10.1016/j.rbmo.2013.12.011

100. Stanic AK, Kim M, Styer AK, Rueda BR. Dendritic cells attenuate the early establishment of endometriosis-like lesions in a murine model. Reprod Sci. 2014;21(10):1228–36.

101. Izumi G, Koga K, Takamura M, Makabe T, Nagai M, Urata Y, et al. Mannose receptor is highly expressed by peritoneal dendritic cells in endometriosis. Fertil Steril [Internet]. 2017;107(1):167-173.e2. Available from:

http://dx.doi.org/10.1016/j.fertnstert.2016.09.036

102. Peter H, Pichler W, Albert M, Albrecht K, Ballmer-weber B, Baum C, et al. Klinische Immunologie. 3. Auflage. Elsevier GmbH München; 2012.

103. Milewski Ł, Dziunycz P, Barcz E, Radomski D, Roszkowski PI, Korczak-Kowalska G, et al. Increased levels of human neutrophil peptides 1, 2, and 3 in peritoneal fluid of patients with endometriosis: Association with neutrophils, T cells and IL-8. J Reprod Immunol. 2011;91(1–2):64–70.

104. Arici A. Local cytokines in endometrial tissue: The role of interleukin-8 in the pathogenesis of endometriosis. Ann N Y Acad Sci. 2002;955:101–9.

105. Takamura M, Koga K, Izumi G, Urata Y, Nagai M, Hasegawa A, et al. Neutrophil depletion reduces endometriotic lesion formation in mice. Am J Reprod Immunol. 2016;76(3):193–8.

106. Na YJ, Yang SH, Baek DW, Lee DH, Kim KH, Choi YM, et al. Effects of peritoneal fluid from endometriosis patients on the release of vascular endothelial growth factor by neutrophils and monocytes. Hum Reprod. 2006;21(7):1846–55.

107. Kim JY, Lee DH, Joo JK, Jin JO, Wang JW, Hong YS, et al. Effects of peritoneal fluid from endometriosis patients on interferon-γ-induced protein-10 (CXCL10) and interleukin-8 (CXCL8) released by neutrophils and CD4+ T cells. Am J Reprod Immunol. 2009;62(3):128–38.

108. Tabibzadeh S. Regulatory Roles of IFN-Gamma in Human Endometrium. Ann N Y Acad Sci. 1994;734(1):1–6.

109. Yeaman GR, Collins JE, Currie JK, Guyre PM, Wira CR, Fanger MW. IFN-γ is produced by polymorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polymorphonuclear neutrophils. J Immunol. 1998;160(10):5145–53.

110. Berkes E, Oehmke F, Tinneberg HR, Preissner KT, Saffarzadeh M. Association of neutrophil extracellular traps with endometriosis-related chronic inflammation. Eur J Obstet Gynecol Reprod Biol [Internet]. 2014;183:193–200. Available from: http://dx.doi.org/10.1016/j.ejogrb.2014.10.040

111. Cho SH, Cho H, Nam A, Kim HY, Choi YS, Park KH, et al. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil Steril [Internet]. 2008;90(6):2073–9. Available from: http://dx.doi.org/10.1016/j.fertnstert.2008.03.061

112. Tokmak A, Yildirim G, Öztaş E, Akar S, Erkenekli K, Gülşen P, et al. Use of Neutrophil-to-Lymphocyte Ratio Combined with CA-125 to Distinguish Endometriomas from Other Benign Ovarian Cysts. Reprod Sci. 2016;23(6):795–802.

113. Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res. 2019;12(1):1–9.

114. Yang H, Lang JH, Zhu L, Wang S, Sha GH, Zhang Y. Diagnostic value of the neutrophil-to-lymphocyte ratio and the combination of serum CA-125 for stages III and IV endometriosis. Chin Med J (Engl). 2013;126(11):2011–4.

115. Kim SK, Park JY, Jee BC, Suh CS, Kim SH. Association of the neutrophil-to-lymphocyte ratio and CA 125 with the endometriosis score. Clin Exp Reprod Med. 2014;41(4):151–7.

116. Yavuzcan A, Çağlar M, Üstün Y, Dilbaz S, Özdemir I, Yildiz E, et al. Evaluation of mean platelet volume, neutrophil/lymphocate ratio and platelet/lymphocate ratio in advanced stage

endoemtriosis with endometrioma. J Turkish Ger Gynecol Assoc. 2013;14(4):210–5.

117. Sivridis E, Giatromanolaki A, Agnantis N, Anastasiadis P. Mast cell distribution and density in the normal uterus - Metachromatic staining using lectins. Eur J Obstet Gynecol Reprod Biol. 2001;98(1):109–13.

118. Mori A, Zhai YL, Toki T, Nikaido T, Fujii S. Distribution and heterogeneity of mast cells in the human uterus. Hum Reprod. 1997;12(2):368–72.

119. Fujiwara H, Konno R, Netsu S, Sugamata M, Shibahara H, Ohwada M, et al. Localization of mast cells in endometrial cysts. Am J Reprod Immunol. 2004;51(5):341–4.

120. Drudy L, Sheppard B, Bonnar J. Mast cells in the normal uterus and in dysfunctional uterine bleeding. Eur J Obstet Gynecol Reprod Biol. 1991;39(3):193–201.

121. Zhang J, Nie G, Jian W, Woolley DE, Salamonsen LA. Mast cell regulation of human endometrial matrix metalloproteinases: A mechanism underlying menstruation. Biol Reprod. 1998;59(3):693–703.

122. Ludwig H, Spornitz UM. Microarchitecture of the Human Endometrium by Scanning Electron Microscopy: MEnstrual Desquamation and Remodeling. Ann N Y Acad Sci. 1991;622:28–46.

123. Menzies FM, Shepherd MC, Nibbs RJ, Nelson SM. The role of mast cells and their mediators in reproduction, pregnancy and labour. Hum Reprod Update. 2011;17(3):383–96.

124. Varayoud J, Ramos JG, Bosquiazzo VL, Muñoz-de-Toro M, Luque EH. Mast cells degranulation affects angiogenesis in the rat uterine cervix during pregnancy. Reproduction. 2004;127(3):379–87.

125. Guo CB, Kagey-Sobotka A, Lichtenstein LM, Bochner BS. Immunophenotyping and functional analysis of purified human uterine mast cells. Blood. 1992;79(3):708–12.

126. Matsuzaki S, Canis M, Darcha C, Fukaya T, Yajima A, Bruhat MA. Increased mast cell density in peritoneal endometriosis compared with eutopic endometrium with endometriosis. Am J Reprod Immunol. 1998;40(4):291–4.

127. Sugamata M, Ihara T, Uchiide I. Increase of activated mast cells in human endometriosis. Am J Reprod Immunol. 2005;53(3):120–5.

128. Kempuraj D, Papadopoulou N, Stanford EJ, Christodoulou S, Madhappan B, Sant GR, et al. Increased numbers of activated mast cells in endometriosis lesions positive for corticotropin-releasing hormone and urocortin. Am J Reprod Immunol. 2004;52(4):267–75.

129. Anaf V, Chapron C, El Nakadi I, De Moor V, Simonart T, Noël JC. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. Fertil Steril. 2006;86(5):1336–43.

130. Paula R, Oliani AH, Vaz-Oliani DCM, D'Ávila SCGP, Oliani SM, Gil CD. The intricate role of mast cell proteases and the annexin A1-FPR1 system in abdominal wall endometriosis. J Mol Histol. 2015;46(1):33–43.

131. Konno R, Yamada-Okabe H, Fujiwara H, Uchiide I, Shibahara H, Ohwada M, et al. Role of immunoreactions and mast cells in pathogenesis of human endometriosis--morphologic study and gene expression analysis. Hum cell Off J Hum Cell Res Soc. 2003;16(3):141–9.

132. Jeziorska M, Salamonsen LA, Woolley DE. Mast cell and eosinophil distribution and activation in human endometrium throughout the menstrual cycle. Biol Reprod. 1995;53(2):312–20.

133. Ramirez GA, Yacoub M-R, Ripa M, Mannina D, Cariddi A, Saporiti N, et al. Eosinophils from

Physiology to Disease: A Comprehensive Review. Biomed Res Int [Internet]. 2018;2018:9095275. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29619379

134. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: Characteristics and functions. Front Med. 2017;4(JUL):1–6.

135. Stone KD, Prussin C, Metcalfe DD. IgE, Mast cells, Basophils, and Eosinophils. J Allergy Clin Immunol [Internet]. Dec;125(2 Suppl 2):S73-80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091674907017277

136. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. J Allergy Clin Immunol [Internet]. 2018;142(2):370–80. Available from: https://doi.org/10.1016/j.jaci.2017.10.042

137. Davis ME, Hulit BE. The menstrual cycle: Changes in circulating eosinophils in women during the menstrual cycle and reproduction. Obstet Gynecol Surv. 1950;5(2):233–4.

138. Pepper H, Lindsay S. Levels of Eosinophils, Platelets, Leukocytes and 17-Hydroxycorticosteroids During Normal Menstrual Cycle. Proc Soc Exp Biol Med. 1960;104(1):145–7.

139. Blumenthal RD, Samoszuk M, Taylor AP, Brown G, Alisauskas R, Goldenberg DM. Degranulating eosinophils in human endometriosis. Am J Pathol [Internet]. 2000;156(5):1581–8. Available from: http://dx.doi.org/10.1016/S0002-9440(10)65030-4

140. Eidukaite A, Tamosiunas V. Activity of eosinophils and immunoglobulin E concentration in the peritoneal fluid of women with endometriosis. Clin Chem Lab Med. 2004;42(6):590–4.

141. Hornung D, Dohrn K, Sotlar K, Greb RR, Wallwiener D, Kiesel L, et al. Localization in tissues and secretion of eotaxin by cells from normal endometrium and endometriosis. J Clin Endocrinol Metab. 2000;85(7):2604–8.

142. Lebien TW, Tedder TF. B lymphocytes: How they develop and function. Blood. 2008;112(5):1570–80.

143. Eibel H, Kraus H, Sic H, Kienzler A-K, Rizzi M. B cell biology: an overview. Curr Allergy Asthma Rep [Internet]. 2014 May;14(5):434. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24633618

144. Mettler L, Volkov NI, Kulakov VI, Jürgensen A, Parwaresch MR. Lymphocyte Subsets in the Endometrium of Patients with Endometriosis Throughout the Menstrual Cycle. Am J Reprod Immunol. 1996;36(6):342–8.

145. Berbic M, Ng CHM, Black K, Markham R, Russell P, Basten A, et al. A novel pilot study of endometrial stromal cells and immune cell populations in sentinel uterine-draining lymph nodes during the menstrual cycle and in endometriosis. Reprod Sci. 2013;20(11):1339–48.

146. Oertelt-Prigione S. Immunology and the menstrual cycle. Autoimmun Rev [Internet]. 2012;11(6–7):A486–92. Available from: http://dx.doi.org/10.1016/j.autrev.2011.11.023

147. Peeva E, Venkatesh J, Diamond B. Tamoxifen Blocks Estrogen-Induced B Cell Maturation but Not Survival. J Immunol. 2005;175(3):1415–23.

148. Witz CA, Montoya IA, Dey TD, Schenken RS. Characterization of lymphocyte subpopulations and T cell activation in endometriosis. Am J Reprod Immunol. 1994;32(3):173–9.

149. Klentzeris LD, Bulmer JN, Liu DT, Morrison L. Endometrial leukocyte subpopulations in women with endometriosis. Eur J Obstet Gynecol Reprod Biol [Internet]. 1995 Nov [cited

2019 May 24];63(1):41–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8674564

150. Gebel HM, Braun DP, Rotman C, Rana N, Dmowski WP. Mitogen induced production of polyclonal IgG is decreased in women with severe endometriosis. Am J Reprod Immunol. 1993;29(2):124–30.

151. Oosterlynck DJ, Cornillie FJ, Waer M, Koninckx PR. Immunohistochemical characterization of leucocyte subpopulations in endometriotic lesions. Arch Gynecol Obstet. 1993;253(4):197–206.

152. Antsiferova YS, Sotnikova NY, Posiseeva L V., Shor AL. Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril. 2005;84(6):1705–11.

153. Badawy SZA, Cuenca V, Kaufman L, Stitzel A, Thompson M. The regulation of immunoglobulin production by B cells in patients with endometriosis. Fertil Steril [Internet]. 1989;51(5):770–3. Available from: http://dx.doi.org/10.1016/S0015-0282(16)60664-7

154. Chishima F, Hayakawa S, Hirata Y, Nagai N, Kanaeda T, Tsubata K, et al. Peritoneal and peripheral B-1-cell populations in patients with endometriosis. J Obstet Gynaecol Res. 2000;26(2):141–9.

155. Hever A, Roth RB, Hevezi P, Marin ME, Acosta JA, Acosta H, et al. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. Proc Natl Acad Sci U S A. 2007;104(30):12451–6.

156. Walankiewicz M, Grywalska E, Polak G, Korona-Glowniak I, Witt E, Surdacka A, et al. The Increase of Circulating PD-1- and PD-L1-Expressing Lymphocytes in Endometriosis: Correlation with Clinical and Laboratory Parameters. Mediators Inflamm [Internet]. 2018;2018:7041342. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30595667

157. Odukoya OA, Bansal A, Wilson AP, Weetman AP, Cooke ID. Endometriosis: Serum-soluble CD23 in patients with endometriosis and the effect of treatment with danazol and leuprolide acetate depot injection. Hum Reprod. 1995;10(4):942–6.

158. Sur LM, Floca E, Sur DG, Colceriu M, Gabriel S, Sur G. Antinuclear Antibodies: Marker of Diagnosis and Evolution in Autoimmune Diseases. Lab Med. 2018;49(3):316–22.

159. Cooper AM, Hobson PS, Jutton MR, Kao MW, Drung B, Schmidt B, et al. Soluble CD23 Controls IgE Synthesis and Homeostasis in Human B Cells. J Immunol. 2012;188(7):3199–207.

160. Riccio LGC, Jeljeli M, Santulli P, Chouzenoux S, Doridot L, Nicco C, et al. B lymphocytes inactivation by Ibrutinib limits endometriosis progression in mice. Hum Reprod. 2019;34(7):1225–34.

161. Kim JS, Choi YS, Park JH, Yun J, Kim S, Lee JH, et al. Role of B-cell translocation gene 1 in the pathogenesis of endometriosis. Int J Mol Sci. 2019;20(13):1–13.

162. Fernàndez-shaw S, Hicks BR, Yudkin PL, Kennedy S, Barlow DH, Starkey PM. Anti-endometrial and anti-endothelial auto-antibodies in women with endometriosis. Hum Reprod. 1993;8(2):310–5.

163. Fernandéz-Shaw S, Kennedy SH, Hicks BR, Edmonds K, Starkey PM, Barlow DH. Anti-endometrial antibodies in women measured by an enzyme-linked immunosorbent assay. Hum Reprod. 1996;11(6):1180–4.

164. Wild RA, Shivers CA. Antiendometrial antibodies in patients with endometriosis. Am J

Reprod Immunol Microbiol. 1985;8(3):84–6.

165. Palacio JR, Iborra A, Gris JM, Andolz P, Martínez P. Anti-endometrial autoantibodies in women with a diagnosis of infertility. Am J Reprod Immunol. 1997;38(2):100–5.

166. Sarapik A, Haller-Kikkatalo K, Utt M, Teesalu K, Salumets A, Uibo R. Serum anti-endometrial antibodies in infertile women - potential risk factor for implantation failure. Am J Reprod Immunol. 2010;63(5):349–57.

167. Ozhan E, Kokcu A, Yanik K, Gunaydin M. Investigation of diagnostic potentials of nine different biomarkers in endometriosis. Eur J Obstet Gynecol Reprod Biol [Internet]. 2014;178:128–33. Available from: http://dx.doi.org/10.1016/j.ejogrb.2014.04.037

168. Gajbhiye R, Suryawanshi A, Khan S, Meherji P, Warty N, Raut V, et al. Multiple endometrial antigens are targeted in autoimmune endometriosis. Reprod Biomed Online [Internet]. 2008;16(6):817–24. Available from: http://dx.doi.org/10.1016/S1472-6483(10)60147-2

169. Paul S, Lal G. Role of gamma-delta (gd) T cells in autoimmunity. 2015;97(February):259–71.

170. Ota H, Igarashi S, Tanaka T. Expression of γδT cells and adhesion molecules in endometriotic tissue in patients with endometriosis and adenomyosis. Am J Reprod Immunol. 1996;35(5):477–82.

171. Wu L, Van Kaer L. Natural killer T cells in health and disease. Front Biosci (Schol Ed) [Internet]. 2011;3(1):236–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21196373

172. Lagana AS, Triolo O, Maria F, Granese R, Italo V, Helena P, et al. Natural Killer T cell subsets in eutopic and ectopic endometrium : a fresh look to a busy corner. 2016;941–9.

173. Eidukaite A, Siaurys A, Tamosiunas V. Aberrant expression of CD95 and CD69 molecules among CD56+ cells in women with endometriosis. Am J Reprod Immunol. 2006;55(4):276–81.

174. Josefowicz SZ, Lu L, Rudensky AY. Regulatory T Cells: Mechanisms of Differentiation and Function. Annu Rev Immunol. 20182;(234):531–64.

175. Gogacz M, Winkler I, Bojarska-Junak A, Tabarkiewicz J, Semczuk A, Rechberger T, et al. T regulatory lymphocytes in patients with endometriosis. Mol Med Rep. 2014;10(2):1072–6.

176. Hanada T, Tsuji S, Nakayama M, Wakinoue S, Kasahara K, Kimura F, et al. Suppressive regulatory T cells and latent transforming growth factor-β-expressing macrophages are altered in the peritoneal fluid of patients with endometriosis. Reprod Biol Endocrinol. 2018;16(1):1–8.

177. Hudson QJ, Ashjaei K, Perricos A, Kuessel L, Husslein H, Wenzl R, et al. Endometriosis Patients Show an Increased M2 Response in the Peritoneal CD14+low/CD68+low Macrophage Subpopulation Coupled with an Increase in the T-helper 2 and T-regulatory Cells. Reprod Sci. 2020;27(10):1920–31.

178. Takamura M, Koga K, Izumi G, Hirata T, Harada M, Hirota Y, et al. Simultaneous Detection and Evaluation of Four Subsets of CD4+ T Lymphocyte in Lesions and Peripheral Blood in Endometriosis. Am J Reprod Immunol. 2015;74(6):480–6.

179. Tao J, Cheng M, Tang J, Liu Q, Pan F, Li X. Foxp3 , Regulatory T Cell , and Autoimmune Diseases. Inflammation. 2017;40(1):328–39.

180. Berbic M, Hey-Cunningham AJ, Ng C, Tokushige N, Ganewatta S, Markham R, et al. The role of Foxp3+ regulatory T-cells in endometriosis: A potential controlling mechanism for a

complex, chronic immunological condition. Hum Reprod. 2010;25(4):900–7.

181. Basta P, Majka M, Jozwicki W, Lukaszewska E, Knafel A, Grabiec M, et al. The frequency of CD25+CD4+ and FOXP3+ regulatory T cells in ectopic endometrium and ectopic decidua. Reprod Biol Endocrinol [Internet]. 2010 Oct 5;8:116. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20923543

182. Podgaec S, Rizzo LV, Fernandes LFC, Baracat EC, Abrao MS. CD4+CD25highFoxp3+ Cells Increased in the Peritoneal Fluid of Patients with Endometriosis. Am J Reprod Immunol. 2012;68(4):301–8.

183. Berbic M, Fraser IS. Regulatory T cells and other leukocytes in the pathogenesis of endometriosis. J Reprod Immunol [Internet]. 2011;88(2):149–55. Available from: http://dx.doi.org/10.1016/j.jri.2010.11.004

184. Olkowska-Truchanowicz J, Bocian K, Maksym RB, Białoszewska A, Włodarczyk D, Baranowski W, et al. CD4+ CD25+ FOXP3+ regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. Hum Reprod. 2013;28(1):119–24.

185. Chen S, Zhang J, Huang C, Lu W, Liang Y, Wan X. Expression of the T regulatory cell transcription factor FoxP3 in peri-implantation phase endometrium in infertile women with endometriosis. Reprod Biol Endocrinol. 2012;10:1–7.

186. Budiu RA, Diaconu I, Chrissluis R, Dricu A, Edwards RP, Vlad AM. A conditional mouse model for human MUC1-positive endometriosis shows the presence of anti-MUC1 antibodies and Foxp3+ regulatory T cells. DMM Dis Model Mech. 2009;2(11–12):593–603.

187. Li MQ, Wang Y, Chang KK, Meng YH, Liu LB, Mei J, et al. CD4+Foxp3+ regulatory T cell differentiation mediated by endometrial stromal cell-derived TECK promotes the growth and invasion of endometriotic lesions. Cell Death Dis. 2014;5(10).

188. Liu Q, Ma P, Liu L, Ma G, Ma J, Liu X, et al. Evaluation of PLGA containing anti-CTLA4 inhibited endometriosis progression by regulating CD4 + CD25 + Treg cells in peritoneal fluid of mouse endometriosis model. Eur J Pharm Sci [Internet]. 2017;96:542–50. Available from: http://dx.doi.org/10.1016/j.ejps.2016.10.031

189. Wang XQ, Zhou WJ, Luo XZ, Tao Y, Li DJ. Synergistic effect of regulatory T cells and proinflammatory cytokines in angiogenesis in the endometriotic milieu. Hum Reprod. 2017;32(6):1304–17.

190. Bellelis P, Barbeiro DF, Rizzo LV, Baracat EC, Abrão MS, Podgaec S. Transcriptional changes in the expression of chemokines related to natural killer and T-regulatory cells in patients with deep infiltrative endometriosis. Fertil Steril. 2013;99(7):1987–93.

191. Wei C, Mei J, Tang L, Liu Y, Li D, Li M, et al. 1-Methyl-tryptophan attenuates regulatory T cells differentiation due to the inhibition of estrogen-IDO1-MRC2 axis in endometriosis. Cell Death Dis. 2016;7(12):1–15.

192. Khan KN, Yamamoto K, Fujishita A, Muto H, Koshiba A, Kuroboshi H, et al. Differential Levels of Regulatory T Cells and T-Helper-17 Cells in Women with Early and Advanced Endometriosis. J Clin Endocrinol Metab. 2019;104(10):4715–29.

193. Ho HN, Wu MY, Chao KH, Chen C Der, Chen SU, Yang YS. Peritoneal interleukin-10 increases with decrease in activated CD4+ T lymphocytes in women with endometriosis. Hum Reprod. 1997;12(11):2528–33.

194. Becker JL, Widen RH, Mahan CS, Yeko TR, Parsons AK, Spellacy WN. Human peritoneal

macrophage and T lymphocyte populations in mild and severe endometriosis. Am J Reprod Immunol. 1995;34(3):179–87.

195. Podgaec S, Dias Junior JA, Chapron C, Oliveira RM de, Baracat EC, Abrão MS. Th1 and Th2 immune responses related to pelvic endometriosis. Rev Assoc Med Bras. 2010;56(1):92–8.

196. Riccio L da GC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol [Internet]. 2018 Jul [cited 2019 May 24];50:39–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29506962

197. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today. 1993 Jul;14(7):353–6.

198. Chen P, Zhang Z, Chen Q, Ren F, Li T, Zhang C, et al. Expression of Th1 and Th2 cytokine-associated transcription factors, T-bet and GATA-3, in the eutopic endometrium of women with endometriosis. Acta Histochem [Internet]. 2012;114(8):779–84. Available from: http://dx.doi.org/10.1016/j.acthis.2012.01.005

199. Borthwick LA, Wynn TA, Fisher AJ. Cytokine mediated tissue fibrosis. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2013 Jul;1832(7):1049–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0925443912002268

200. Gogacz M, Winkler I, Bojarska-Junak A, Tabarkiewicz J, Semczuk A, Rechberger T, et al. Increased percentage of Th17 cells in peritoneal fluid is associated with severity of endometriosis. J Reprod Immunol [Internet]. 2016;117:39–44. Available from: http://dx.doi.org/10.1016/j.jri.2016.04.289

201. Osuga Y, Hirota Y, Hirata T, Takamura M, Urata Y, Harada M, et al. Th2 cells and Th17 cells in the development of endometriosis – possible roles of interleukin-4 and interleukin-17A. Vol. 8, Journal of Endometriosis. Wichtig Publishing Srl; 2016. p. 136–40.

202. Zhang X, Xu H, Lin J, Qian Y, Deng L. Peritoneal fluid concentrations of interleukin-17 correlate with the severity of endometriosis and infertility of this disorder. BJOG [Internet]. 2005 Aug;112(8):1153–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16045534

203. Andreoli CG, Genro VK, Souza CA, Michelon T, Bilibio JP, Scheffel C, et al. T helper (Th)1, Th2, and Th17 interleukin pathways in infertile patients with minimal/mild endometriosis. Fertil Steril. 2011;95(8):2477–80.

204. Slabe N, Meden-Vrtovec H, Verdenik I, Kosir-Pogacnik R, Ihan A. Cytotoxic T-cells in peripheral blood in women with endometriosis. Geburtshilfe Frauenheilkd. 2013;73(10):1042–8.

205. Ota H, Matsui T, Igarashi S, Tanaka H, Hayakawa M, Tanaka T. Effect of danazol on the immunocompetent cells in the eutopic endometrium in patients with endometriosis: A multicenter cooperative study. Fertil Steril [Internet]. 1996;65(3):545–51. Available from: http://dx.doi.org/10.1016/S0015-0282(16)58151-5