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Immunological changes associated with adenomyosis: a systematic review

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BACKGROUND: Adenomyosis is a benign gynecological disorder associated with subfertility, pelvic pain and abnormal uterine bleeding that have significant consequences for the health and quality of life of women. Histologically, it is defined as the presence of ectopic endometrial islets within the myometrium. Its pathogenesis has not yet been elucidated and several pieces of the puzzle are still missing. One process involved in the development of adenomyosis is the increased capacity of some endometrial cells to infiltrate the myometrium. Moreover, the local and systemic immune systems are associated with the onset of the disease and with maintaining it. Numerous observations have highlighted the activation of immune cells and the release of immune soluble factors in adenomyosis. The contribution of immunity occurs in conjunction with hormonal aberrations and activation of the epithelial to mesenchymal transition (EMT) pathway, which promotes migration of endometrial cells. Here, we review current knowledge on the immunological changes in adenomyosis, with the aim of further elucidation of the pathogenesis of this disease.

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OBJECTIVE AND RATIONALE: The objective was to systematically review the literature regarding the role of the immune system in development of adenomyosis in the inner and the outer myometrium, in humans.

SEARCH METHODS: A systematic review of published human studies was performed in MEDLINE, EMBASE and Cochrane Library databases from 1970 to February 2019 using the combination of Medical Subject Headings (MeSH): Adenomyosis AND ('Immune System' OR 'Gonadal Steroid Hormones'), and free-text terms for the following search terms (and their variants): Adenomyosis AND (immunity OR immune OR macrophage OR 'natural killer cell' OR lymphocyte* OR leucocyte* OR HLA OR inflammation OR 'sex steroid' OR 'epithelial to mesenchymal transition' OR 'EMT'). Studies in which no comparison was made with control patients, without adenomyosis (systemic sample and/or eutopic endometrium), were excluded.

OUTCOMES: A total of 42 articles were included in our systematic review. Changes in innate and adaptive immune cell numbers were described in the eutopic and/or ectopic endometrium of women with adenomyosis compared to disease-free counterparts. They mostly described an increase in lymphocyte and macrophage cell populations in adenomyosis eutopic endometrium compared to controls. These observations underscore the immune contributions to the disease pathogenesis. Thirty-one cytokines and other markers involved in immune pathways were studied in the included articles. Pro-inflammatory cytokines (interleukin (IL) 6, IL1β, interferon (IFN) α, tumor necrosis factor α, IFNγ) as well as anti-inflammatory or regulatory mediators (IL10, transforming growth factor β…) were found to be elevated in the eutopic endometrium and/or in the ectopic endometrium of the myometrium in women with adenomyosis compared to controls. Moreover, in women affected by adenomyosis, immunity was reported to be directly or indirectly linked to sex steroid hormone aberrations (notably changes in progesterone receptor in eutopic and ectopic endometrium) in three studies and to EMT in four studies.

WIDER IMPLICATIONS: The available literature clearly depicts immunological changes that are associated with adenomyosis. Both systemic and local immune changes have been described in women affected by adenomyosis, with the coexistence of changes in inflammatory as well as anti-inflammatory signals. It is likely that these immune changes, through an EMT mechanism, stimulate the migration of endometrial cells into the myometrium that, together with an endocrine imbalance, promote this inflammatory process. In light of the considerable impact of adenomyosis on women's health, a better understanding of the role played by the immune system in adenomyosis is likely to yield new research opportunities to better understand its pathogenesis.

Key words: adenomyosis / immune system / innate immunity / adaptive immunity / pathogenesis / sex steroid hormones / epithelial to mesenchymal transition / symptoms / diffuse adenomyosis / focal adenomyosis

Introduction

Adenomyosis is defined by the ectopic presence of endometrial islets in the myometrium (Bird *et al.*, 1972; Bergeron *et al.*, 2006; Benagiano et al., 2012). This gynecological disorder is clinically characterized by dysmenorrhea, dyspareunia, abnormal uterine bleeding (AUB) and subfertility (Ferrero *et al.*, 2009; Naftalin *et al.*, 2014; Vercellini *et al.*, 2014). Estimates of the prevalence are hard to obtain and are probably underestimated. However, prevalence is thought to be in the order of 20–30% at hysterectomy (Taran *et al.*, 2013) and it can be higher with in-depth histological examination and with imaging tools for the diagnosis in non-operated women (Pinzauti *et al.*, 2015; Chapron *et al.*, 2020). Furthermore, the latter examinations have led to the realization that adenomyosis is a heterogeneous and widespread disease that includes distinct forms according to the location of the ectopic lesions in the myometrium, and whether diffuse and/or focal in the inner and/or outer myometrium (Chapron *et al.*, 2020).

Several theories for its pathogenesis have been proposed. Both human and experimental studies favor the theory of invasion of the myometrium by endometrial cells, although the *de novo* development of adenomyosis from Müllerian rests (microscopic structures, stemmed from Müllerian ducts that is the embryologic origin of the female genital organ) or stem cells is a possibility (Ferenczy, 1998). According to the most widely accepted theory, adenomyosis lesions originate from the basal layer of the endometrium that deeply invaginate between smooth muscle cell bundles into the myometrium (Bergeron *et al.*, 2006). This increased capacity of migration and invasiveness of adenomyosis stromal cells has been observed in several experimental studies (Chen *et al.*, 2010a; Mehasseb *et al.*, 2010; Wang *et al.*, 2014; Zhou *et al.*, 2018). Moreover, those capacities are enhanced by immune mediators (Chen *et al.*, 2010a; Wang *et al.*, 2014). It has been proposed that invaginations occur due to a weakened myometrium a result of tissue trauma or a predisposition of the myometrium, thereby enabling the endometrial tissue to migrate (Uduwela *et al.*, 2000).

The diagnostic accuracy of imaging adds further complexity to our understanding of the pathogenesis of adenomyosis given that a number of previous studies have suggested that the pathogenic mechanism of adenomyosis is specific to the location of the lesion (Chapron *et al.*, 2017; Kishi *et al.*, 2017; Khan *et al.*, 2019). Ultimately, the development of adenomyosis lesions involves several pathogenic mechanisms (Vannuccini *et al.*, 2017), and notably specific immune changes (Ota *et al.*, 1998).

Indeed, an abundance of data from diverse sources highlights the existence of aberrant immune responses in women with adenomyosis. At the physiological level, an efficient immune response requires a highly balanced interactive network of effector immune cells, regulatory mechanisms and immune soluble factors. Moreover, in the uterus, the organization of the immune system serves a dual purpose. On the one hand, it is essential to protect against pathogen invasion by way of an appropriate inflammatory reaction, while on the other hand, a shift toward an immunosuppressive state is also required to allow successful embryonic implantation. These separate functions involve both the innate and adaptive components of the immune system. In adenomyosis, a series of immune responses are activated, including changes in both cellular and humoral immunity, i.e. a change in the cytokines that are released and in the levels of macrophages, lymphocytes and

other immune cells. Additionally, it has been shown that the immune system interacts with other pathogenic mechanisms also described in adenomyosis, such as hormonal modifications (notably, changes in estradiol and progesterone receptors in eutopic and ectopic endometrium from women with adenomyosis (Takahashi *et al.*, 1989; Urabe *et al.*, 1989; Kitawaki *et al.*, 1997, 2001; Ezaki *et al.*, 2001; Chen *et al.*, 2010b; Nie *et al.*, 2010a,b; Mehasseb *et al.*, 2011)) or the activation of epithelial to mesenchymal transition (EMT) signals, in the pathogenesis and the physiopathology of adenomyosis (Chen *et al.*, 2010b; KHan *et al.*, 2015; An *et al.*, 2017a).

In this review, we provide a comprehensive summary of the increasing literature available regarding immune changes described in adenomyosis, including the link with hormonal changes and an EMT, in order to obtain new insights and to more precisely determine the role of immunity in this disease.

Methods

This review was carried out and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA checklist was used to generate this review.

Literature search

PubMed, Embase, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews were searched for relevant published materials. The search strategy was limited to articles published in English involving human subjects between 1970 and February 2019 for all of the indicated electronic databases. The research was performed in association with the referral Inter-University Library of Medicine of Paris Descartes, Paris 5, France. The final searches for relevant studies were performed on the 15 February 2019 using a combination of Medical Subject Headings (MeSH): Adenomyosis AND ('Immune System' OR 'Gonadal Steroid Hormones'), and free-text terms for the following search terms (and their variants): Adenomyosis AND (immunity OR immune OR macrophage OR 'natural killer cell' OR 'lymphocyte' OR 'leucocyte' OR HLA OR inflammation OR 'sex steroid' OR 'epithelial to mesenchymal transition' OR 'EMT'). Potentially relevant articles were examined, and their reference lists were checked in order to identify additional potentially relevant studies.

Eligibility criteria and quality assessment of the included studies

Only published original research articles, in English, such as case–control or cohort studies, prospective clinical trials and involving humans that investigated the presence of immune factors in adenomyotic patients were included. The adenomyosis form had been specified (for example as either diffuse and/or focal within the myometrium) or not specified in the included studies. Case reports or studies that did not compare women with adenomyosis to controls without adenomyosis, or that only compared them to women with endometriosis were excluded. The quality assessment of the included studies was performed by two independent reviewers: the Newcastle–Ottawa scale was used to assess the methodological quality of the included studies (Stang, 2010).

Results

Study characteristics and assessment of the risk of bias

The literature search (Fig. 1), based on our predefined key search items, identified 778 publications after the removal of duplicates. The titles of these publications were screened and assessed for eligibility, resulting in 64 full-text articles deemed to be included in the review. Of these, after evaluation of the full text of the publications, 22 studies were excluded. Ultimately, a total of 42 studies were included in the present systematic review.

The methodological quality of the included studies was assessed using the Newcastle–Ottawa scale for possible sources of bias (Table I). The studies were case–controls and cannot exclude selection bias of the study participants. In addition, the control population was heterogeneous among the included studies, and most of the studies included women with associated comorbidities (e.g. leiomyomas, infertility, ovarian cysts). Only a limited number of the included studies were controlled for possible confounders such as age, parity or associated comorbidities. Thirty-three studies used histology to diagnose adenomyosis, four studies used imaging techniques (MRI or ultrasonography) and five did not specify the tools used to diagnose adenomyosis (in these five studies we assumed that the diagnosis was based on histology, given that the women in the adenomyosis group underwent hysterectomy and/or immunostaining was performed on uterine tissues samples) (Supplementary Table SI). In addition, only 15 studies specified whether the adenomyosis was associated with endometriosis in the study groups: six stated that the women with adenomyosis could have associated endometriosis, and nine stated that the presence of an associated endometriosis in the women with adenomyosis was a factor for exclusion) (Supplementary Table SI). Twenty-seven studies did not specify the endometriosis status in the women with adenomyosis. The types of adenomyosis lesions exhibited by adenomyosis-affected women were specified in 11 of the included studies. The adenomyosis lesions were described as either diffuse or focal within the myometrium. In terms of the phase cycle, 26 of the studies had included women in the proliferative and secretory phase, five had only included women in the proliferative phase and seven in the secretory phase. Four studies did not provide information regarding the phase cycle. Seventeen studies specified that no hormonal therapy was used at the time the blood or uterine samples were collected.

Involvement of innate and adaptive immunity in adenomyosis

Systemic changes.

In seven studies, immune perturbations were described at the systemic level in women with adenomyosis compared to control women (Ota *et al.*, 1992; Yang *et al.*, 2004; Xiaoyu *et al.*, 2013; Gui *et al.*, 2014; Fan *et al.*, 2017; Streuli *et al.*, 2017; Bourdon *et al.*, 2018) (Table II). Two articles compared the immune cells in peripheral blood of women with adenomyosis versus controls. While one study reported no significant differences in terms of the proportion of T cells and natural killer (NK) cells between the women with adenomyosis and the controls (Yang *et al.*, 2004), one described an imbalance between circulating T helper 17 (Th17) and regulatory T cells (Tregs), with a high level of

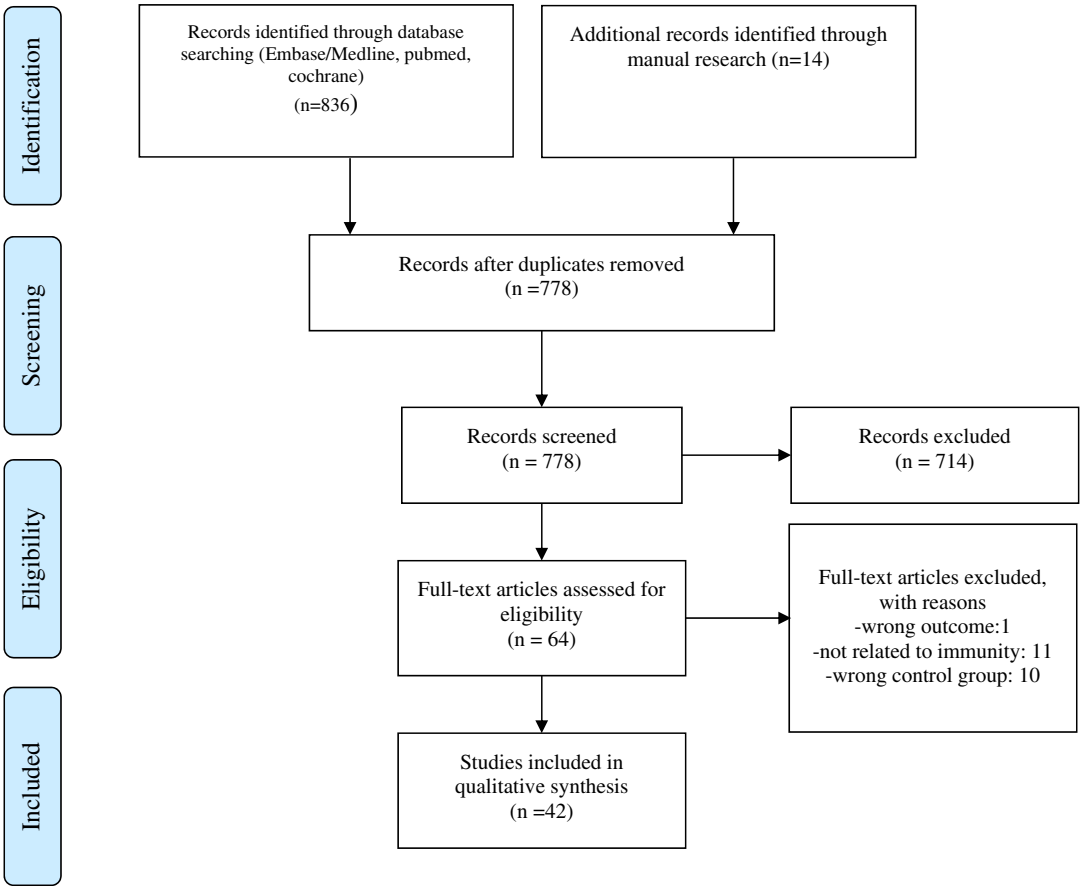

Figure 1. PRISMA flow diagram for the systematic review.

Th17 cells in the women with adenomyosis compared with the control women and a relatively low level of Tregs (Gui *et al.*, 2014). Th17 cells are a subset of CD4+ T-lymphocytes that exert a key role in the host response to fungi and bacteria as well as in the pathogenesis of human immune-mediated diseases. In the Gui *et al.* (2014) study, the Th17/Tregs ratio was higher in the adenomyosis samples compared to the controls during the mid-to-late proliferative phase of the menstrual cycle in women with diffuse or focal adenomyosis lesions. Furthermore, the Th17/Tregs ratio correlated positively with the CA-125 level and with severity of the dysmenorrhea measured using a visual analogic scale score (Gui *et al.*, 2014). No measurements were taken during the secretory phase, and the mechanism underlying the shift in the Th17/Tregs ratio in adenomyosis remains to be elucidated. In the same study, the authors found higher concentrations of the Th17 type interleukin (IL)17A and IL6, which exert potent pro-inflammatory and chemoattractant effects, enhancing neutrophil migration to sites of inflammation. Other pro-inflammatory factors were found to be deregulated, notably the expression of complement proteins (Ota *et al.*, 1992; Xiaoyu *et al.*, 2013). Ota *et al.* (1992) found increased levels of circulating C3 and C4 complement fragments. In line with these results, a study identified that C1, C3 and C5 and complement factor B were significantly upregulated in the sera of women with adenomyosis compared to the controls (Xiaoyu *et al.*, 2013).

Paradoxically, an increase in soluble factors involved in immune suppression accompanying this inflammatory signature has also been reported in women with adenomyosis. For instance, Gui *et al.* (2014) found higher expression of the anti-inflammatory IL-10 cytokine in focal or diffuse adenomyosis samples compared to the controls. In addition, high serum levels of IL10 and IL37 cytokines, which exert inhibitory effects on the innate and adaptive immune response, along with lower levels of pro-inflammatory cytokines (IL17A, IL23, IL25, IL31 and IL33, and tumor necrosis factor (TNF)) were found in two studies in adenomyosis patients compared to the controls (Fan *et al.*, 2017; Bourdon *et al.*, 2018). Another study found a decrease in osteopontin (OPN) levels, which is a pro-inflammatory cytokine that also has a regulatory function, in focal adenomyosis compared with the controls (Streuli *et al.*, 2017).

These findings indicate that there are effects on and interactions with the immune system in adenomyosis-affected women at the systemic level. On the one hand, an increase in several pro-inflammatory cells (an increase in the Th17 lymphocyte/Tregs cell ratio) or in a number of markers involved in inflammatory processes has been shown while, on the other hand, a number of studies have reported an increase in anti-inflammatory signals. In addition, the extent of immunological systemic changes is proportional to the severity of the clinical symptoms associated with the disease in several studies.

Table I **Assessment of the methodological quality of the included studies using the Newcastle–Ottawa scale.**

		An (2017a)	An (2017b)	Nieand Liu (2016)	Li (2015)	Khan (2015)	Gui (2014)	Tremellen (2012)	Chen (2010a,b)	Yang (2006a)	Yang (2006b)	Yang (2004)	Sotnikova (2002)	Jones (1998)	Ota (1998)	Bulmer (1998)	Ota (1996)	Bourdon (2018)	Liu (2016)	Qi (2015)	Ota (1992)	Khan (2010)	Baka (2011)	Li (2013)	Wang (2008)	Ota et al. (2001)	Cai (2018)	Zhihong (2016)	Wang (2014)	Herndon (2016)	Liu (2011)	Nie (2009)	Tokyol (2009)	Ota (1991)	Xiaoyu (2013)	Fan (2017)	Scheerer (2016)	Ulukus (2005)	Carrarelli (2016)	Ota (1993)	Streuli (2017)	Miyashita (2019)	Wang (2009)
Selection	Is the case definition adequate? a) yes, with independent validation* b) yes, e.g., record linkage or based on self-reports c) no description	*	*	*	*	*	*	*	*	*	*	*	*					*	*		*	*		*	*		*	*	*	*		*	*	*	*		*	*	*	*	*	*
	Representativeness of the cases a) consecutive or obviously representative series of cases* b) potential for selection biases or not stated	*	*	*	*	*	*	*	*	*	*	*	*					*	*	*		*	*	*	*		*	*				*				*	*	*	*	*		*
	Selection of Controls a) community controls* b) hospital controls c) no description																																	*								
	Definition of Controls a) no history of disease (endpoint)* b) no description of source	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*		*	*	*	*	*	*	*	*
Comparability	Comparability of cases and controls on the basis of the design or analysis a) study controls for phase cycle* b) study controls for any additional factor*		*	*	*		*	*		*	*	*	*	*	*	*	*		*	*				*			*	*		*	*	*	*		*			*		*		*
Exposure	Ascertainment of exposure a) secure record (e.g. surgical records, research records)* b) structured interview where blind to case/control status* c) interview not blinded to case/control status d) written self-report or medical record only e) no description	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
	Same method of ascertainment for cases and controls a) yes* b) no	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*		*	*	*		*	*	*	*	*	*	*	*	*	*	*
	Total score	5	6	5	6	5	6	6	5	6	6	6	6	3	4	4	4	5	6	5	3	5	4	6	5	3	5	6	3	5	5	4	5	3	7	5	4	6	5	6	5	4	6

Each asterisk (*) denotes one point to assess the quality of included studies. Stars awarded for each quality item serve as a quick visual assessment of the quality of the included studies. The total score corresponds to the total number of stars of each quality item.

Table II Systemic immune changes in women affected by adenomyosis.

First author, Year	Methods used for analysis	Systemic immune changes compared to controls
Gui (2014)	Flow cytometry, rt-qPCR, IHC	• Increase in Th17cells (IL17A), IL6, IL10 and IL17 (diffuse and focal forms) • Decrease in T reg cells (FOXP3) (diffuse and focal forms) • No significant difference for TGFbeta (diffuse and focal forms)
Yang (2004)	Flow cytometry	• Similar proportions of lymphocytes and NK cells
Bourdon (2018)	Multiplex immunoassay	• Decrease in IL17F, IL 23, IL25, IL31 and IL33 in the overall population • Decrease in IL17F in the isolated focal adenomyosis group • Decrease in IL23, IL25, IL31 and IL33 in the associated diffuse adenomyosis group and the focal adenomyosis group • Increase in TNFbeta in the isolated diffuse group compared to the isolated focal adenomyosis group
Ota (1992)	LAPA, flow cytometry	• Increase in complement C3–C4
Xiaoyu (2013)	iTRAQ, WB	• Changes in 25 proteins partly involved in immune responses, inflammation responses, and complement activation (inflammatory response (13%), immune response (13%) and complement activation (11%))
Fan (2017)	ELISA	• Increase in IL-10 and IL-37 • Decrease in IL-17A and TNF
Streuli (2017)	ELISA	• Decrease in OPN levels in the focal adenomyosis group

IHC, immunohistochemistry; IL, interleukin; iTRAQ, isobaric tags for relative and absolute quantitation; LAPA, latex agglutination photometric assay; NK, FOXP3, forkhead box P3; NK: natural killer cells; OPN, osteopontin; RT-qPCR, reverse transcription–quantitative PCR; TGF, transforming growth factor; Th, T helper; TNF, tumor necrosis factor; WB, western blot.

Local modifications

Most of the studies focusing on the role of the immune system in the pathophysiology of adenomyosis investigated local uterine changes, both in eutopic and ectopic endometrial cells of adenomyosis uteri. Alterations in the profile of specific immune cells and the expression of immune mediators were described in adenomyotic tissues.

Macrophages

Macrophages play key roles in both innate and adaptive immune responses as they are involved in phagocytosis, antigen presentation to T cells and the secretion of cytokines/chemokines. In normal endometrium, macrophages represent approximately 10% of the total endometrial immune cell population (Wira *et al.*, 2015). Ten studies assessed the abundance of macrophages in the uterus of women with adenomyosis versus controls by means of CD68 or CD163 expression (Ota *et al.*, 1991; Ota *et al.*, 1996; Bulmer *et al.*, 1998; Jones *et al.*, 1998; Tremellen and Russell, 2012; Nie and Liu, 2016; Scheerer *et al.*, 2016; Zhihong *et al.*, 2016; An *et al.*, 2017a,b) (Table III). Most found an increase in the levels of macrophage markers in eutopic and/or ectopic endometrium compared to controls.

The plasticity of the phenotype and function of macrophages is also a factor in the development and/or the pathophysiology of adenomyosis (An *et al.*, 2017b). Macrophages are classified into classically activated macrophages (M1) with inflammatory and phagocytic properties and alternatively activated (M2) macrophages with anti-inflammatory and tissue remodeling and repair activities (Murray, 2017). There is a paucity of data regarding macrophage activation in adenomyosis. One study, using eutopic endometrial cells from adenomyosis-affected uteri co-cultured with macrophages, showed an altered switch to the M2 phenotype compared to endometrial cells from disease-free uteri (An *et al.*, 2017b). However, in both samples (eutopic endometrial cells from adenomyosis and disease-free uteri) expression of the IL10 gene (which is typically associated with the M2 profile) increased after *in vitro* co-culture with macrophages (An *et al.*, 2017b). Similarly, another study found an increase in CD163 in eutopic endometrium (a marker of M2 macrophages) in the 'severe' diffuse adenomyosis form, defined as a widespread junctional zone thickening of the majority of the anterior and/or posterior uterine wall, compared to controls (Tremellen and Russell, 2012). Further studies of the role of macrophages in the physiopathology of adenomyosis are, therefore, needed, given that this dualistic concept of macrophage polarization has recently been challenged as it may not represent the complex *in vivo* environment that macrophages are exposed to Martinez and Gordon (2014).

Correlation between the increase in the number of macrophages and the severity of adenomyosis disease has also been studied (Tremellen and Russell, 2012; Nie and Liu, 2016). A significantly increased macrophage population in eutopic endometrium from women experiencing implantation failure was found in those with 'severe' diffuse adenomyosis (Tremellen and Russell, 2012). However, there was no comparison with women who had not experienced implantation failure. Moreover, the increase in the number of macrophages in eutopic and ectopic endometrium correlated directly and significantly with the severity of dysmenorrhea in another study (Nie and Liu, 2016).

Collectively, these data indicate that the number of macrophages increases in eutopic and ectopic endometrium in adenomyosis uteri. Circulating monocytes leave the blood flow and migrate into tissues for homeostasis and to cause inflammation. They differentiate into macrophages following exposure to local growth factors and pro-inflammatory cytokines (Shapouri-Moghaddam *et al.*, 2018). Of the

Table III Immune cell changes in eutopic and ectopic endometrium from women with adenomyosis.

First author, Year	Methods used	Type of samples	Outcomes—adenomyosis eutopic endometrium compared to controls	Outcomes—adenomyosis ectopic endometrium compared to controls
			Macrophages	
An (2017a)	IHC, WB	Uterine tissue/*in vitro* cell culture	• Increase in CD68* • CD68 markers positively correlated with EMT markers	• Increase in CD68*
An (2017b)	RT-qPCR	Uterine tissue/*in vitro* cell culture	• Endometrial cells co-cultured with macrophages for 2 days (*in vitro*): No increase in CD163 in adenomyosis eutopic endometrial cells, whereas increased in normal endometrial cells	
Nie and Liu (2016)	IHC	Uterine tissue	• Increase in CD68	• Increase in CD68
Tremellen and Russell (2012)	IHC	Uterine tissue	• Higher stromal CD163 density, especially in severe diffuse adenomyosis	
Jones (1998)	IHC	Uterine tissue	• No significant difference for CD68	• No significant difference for CD68
Bulmer (1998)	IHC	Uterine tissue	• No significant differences for CD68	• No significant differences for CD68
Ota (1996)	IHC	Uterine tissue	• Increase in CD68*	• Increase in CD68*
Zhihong (2016)	RT-qPCR, IHC	Uterine tissue	• Increase in CD68	
Ota (1991)	IHC	Uterine tissue		• Increase in CD68*
Scheerer (2016)	IHC	Uterine tissue	• Increase in CD68	• Increase in CD68
			Polymorphonuclear leukocytes	
Sotnikova (2002)	ELISA	*In vitro* cell culture	• Decrease in IL8	• Decrease in IL8
Ulukus (2005)	IHC	Uterine tissue	• Decrease in IL8	• No difference for IL8*
			Natural killer cells	
Tremellen and Russell (2012)	IHC	Uterine tissue	• No differences in CD56 in the overall population • Increase in CD56 in severe diffuse adenomyosis	
Yang (2004)	Flow cytometry	Uterine tissue	• No significant differences for CD56 • Decrease in KIRs NKB1, and GL183	• No significant difference for CD56 • No significant differences for KIRs NKB1, and GL183
Jones (1998)	IHC	Uterine tissue	• No significant difference for CD56	• No significant difference for CD56
			Lymphocytes	
Ota (1996)	IHC	Uterine tissue	• Increase in CD3, CD4, CD8, TCR Beta F1, TCR gamma 1*	• Increase in TCRgamma1* • No difference in CD3, CD4, CD8, TCR Beta F1*
Scheerer (2016)	IHC	Uterine tissue	• Increase in CD3, CD4, CD8	• Increase in CD3, CD4, CD8, CD20
Gui (2014)	RT-qPCR, IHC	Uterine tissue	• Decrease in FoxP3 (diffuse and focal forms)	• Increase in IL17a • Decrease in FoxP3
Yang (2004)	Flow cytometry	Uterine tissue	• No significant differences for CD4, CD8	• No significant differences for CD4, CD8
Jones (1998)	IHC	Uterine tissue	• No significant differences for CD4, CD8	• No significant differences for CD4, CD8
Bulmer (1998)	IHC	Uterine tissue	• No significant differences for CD4, CD8, CD45RA • Increase in CD3 in proliferative phase	• No significant differences for CD3,CD4, CD8, CD45RA

*, no statistical analysis; CD (Cluster of Differentiation) 68, marker for macrophages; CD163, marker for M2 macrophage; CD3, marker for T lymphocyte; CD4, marker for T helper lymphocyte; CD45RA, molecule expressed on all B lymphocyte cells; CD56, marker for NK cells; CD8; marker for T cytotoxic lymphocyte; EMT, epithelial to mesenchymal transition; FoxP3, forkhead box P3, marker of regulatory T cells; IL (interleukin)8, neutrophil chemotactic factor; IL17a, marker for Th17 lymphocytes; KIR, killer cell immunoglobulin-like receptor; NKB1 and GL183 are two KIR subtypes; TCR, T-cell receptor (TCRbetaF1, TCRGamma1 are two TCR subtypes).

multiple functions exerted by these cells, macrophages play an important role in the establishment and maintenance of adenomyosis lesions by driving chronic inflammation, tissue remodeling and defective healing according to their polarization state and in case of uncontrolled activation (Gordon and Martinez, 2010).

Polymorphonuclear leukocytes

Polymorphonuclear leukocytes, also called granulocytes, represent the majority of the circulating white blood cells and they constitute one of the first barriers of defense against pathogens as well as against altered endogenous cells or molecules. They play additional roles in the regulation of innate and adaptive immune responses and tissue homeostasis (Kolaczkowska and Kubes, 2013). In the current literature, aside from the cytokine IL8, which is known to be a neutrophil chemotactic factor (described later in this review), there are no specific data on the role of neutrophils (the most abundant type of granulocyte) in the onset or the progression of adenomyosis (Izumi *et al.*, 2018) (Table III). Moreover, to our knowledge, no data regarding the involvement of other subclasses of granulocytes, such as eosinophils and basophils, in the pathogenesis of adenomyosis have been published.

Natural killer cells

NK cells are a crucial component of the innate immune response and they can rapidly eliminate tumors or infected cells without targeting normal cells (Vivier *et al.*, 2008). This capacity stems from a dynamic equilibrium between various activating and inhibitory signals transmitted by membrane receptors (Björkström *et al.*, 2016). Uterine (u)NK cells are the predominant leukocyte population in human endometrium, comprising 30–40% of the total leukocyte population in the proliferative phase, and their levels increase in the secretory phase as a result of stimulation by IL15, which is a homeostatic cytokine secreted by endometrial stromal cells (Manaster *et al.*, 2008; Wira *et al.*, 2015). uNK cells also play an important role during implantation and trophoblast invasion (Negishi *et al.*, 2018). uNK cells are clearly distinct from peripheral blood NK cells, with distinct microRNA profiles (Koopman *et al.*, 2003; Moffett and Colucci, 2014). Like NK cells in blood, they express CD94, CD56 and CD9, but they do not express CD16, CD8, or CD57. In addition, CD56 is expressed at approximately 10-fold higher levels in uNK cells than in blood NK cells (Yang *et al.*, 2011). Only a few studies have focused on NK cells in the endometrium of adenomyosis patients and controls, and they are based on assessment of the CD56 marker (Jones *et al.*, 1998; Yang *et al.*, 2004; Tremellen and Russell, 2012) (Table III). No significant differences in the level of CD56 in eutopic or ectopic endometrium compared to control endometrium were observed in these studies, except for one, which reported a significant increase in CD56+ NK cells in the eutopic endometrium of women with 'severe' diffuse adenomyosis (Tremellen and Russell, 2012). One study assessed the quantity of killer-cell inhibitory receptors (KIRs) (notably NKB1 and GL183) in eutopic or ectopic endometrium compared to control endometrium (Yang *et al.*, 2004). Their binding to major histocompatibility complex molecules suppresses the cytotoxic activity of the NK cell. The authors found decreased expression of NKB1 and GL183 on NK cells in the eutopic endometrium, thus suggesting a higher degree of NK cell activation, but not in the myometrium, of women with adenomyosis compared to control endometrium (Yang *et al.*, 2004).

In summary, there is no clear evidence of an increase in NK cell numbers in uteri of adenomyosis-affected women, although their activation is increased in eutopic endometrium (Yang *et al.*, 2004). uNK cells are considered to be the dominant immune cells in the uterine mucosa. They are normally inactive during the normal menstrual cycle and are thought to mature into fully functional NK cells during pregnancy. NK cells are also effectors of the innate immune system as a result of their cytolytic and immunomodulatory capabilities. uNK cells and their cell surface receptors, KIRs, are necessary for a successful pregnancy (Alecsandru *et al.*, 2014; Díaz-Peña *et al.*, 2019). Dysfunction of uNK cells plays a role in the first line of the innate immune response or it contributes to abnormal placentation, implantation failure and early pregnancy loss—which is an event described in women with adenomyosis (Moffett and Shreeve, 2015). Numerous studies have demonstrated that adenomyosis is associated with lower rates of successful implantation and an increased risk of early pregnancy loss (Younes and Tulandi, 2017; Horton *et al.*, 2019). The study of the activation of uNK within the endometrium and the myometrium of women with adenomyosis is an area that has great potential for future research of adenomyosis-related reproductive disorders.

HLA molecules

Almost all nucleated cells express HLA class I molecules on their surface, while HLA class II molecules are expressed only by cells specialized in antigen presentation. HLA molecules allow cells to present peptide antigens to T lymphocytes and to thereby elicit or suppress T-cell responses. The level of HLA class I expression on the cell surface is also responsible for protection against NK cell-mediated lysis. Expression of class II antigen is considered to be an important step in the activation of macrophages and/or T cells. Five studies reported alterations in the expression of HLA molecules in adenomyosis (Ota and Igarashi, 1993; Ota *et al.*, 1996; Wang *et al.*, 2008; Baka *et al.*, 2011; Herndon *et al.*, 2016). A study comparing transcriptomes of proliferative eutopic endometrium from women with and without adenomyosis revealed an upregulation of the HLA-DOB|TAP2 gene, which is a gene involved in HLA function (fold change: 2.98) (Herndon *et al.*, 2016). Three studies described an increase in the level of HLA class II molecules (measured by immunohistochemical staining using HLA-DR isotype detection) in the eutopic endometrium of women with adenomyosis (Ota and Igarashi, 1993; Ota *et al.*, 1996; Baka *et al.*, 2011). Two of these studies also reported an increase in HLA class II in the ectopic endometrium, although no indication of the statistical significance was provided (Ota and Igarashi, 1993; Ota *et al.*, 1996). These alterations in terms of the levels of HLA class II molecules reflect the contribution of the immune system in adenomyosis-affected uteri involving inflammatory signals. Another study that assessed the level of HLA class I molecules found no significant difference compared to the controls (Baka *et al.*, 2011). In one study, HLA-G, which is a non-classical major histocompatibility complex class I antigen involved in the establishment and the maintenance of immune tolerance, was found to be highly expressed in eutopic and ectopic endometrial cells from adenomyotic lesions compared to control endometrium (Wang *et al.*, 2008). HLA-G has been described in the literature as a modulator for the activation of key immune cells, such as macrophages, T lymphocytes and NK cells, with the capacity to induce a state of tolerance (Selmani *et al.*, 2009; Ullah *et al.*, 2019).

The findings regarding HLA molecules indicate a chronic local inflammation (illustrated by the increase in HLA-II) along with immune-suppression signals and a process for restoring homeostasis (illustrated by the increase in HLA-G), in adenomyosis-affected uteri, that together facilitate invasion of the myometrium by endometrial cells.

T and B lymphocytes

T (thymus) and B (bone marrow or bursa-derived) cells play key roles in adaptive immune responses. Upon specific antigenic stimulation, these cell types undergo activation and proliferation as part of a specific adaptive immune response with memory features. B cells are responsible for the humoral immune response that involves antibody production, while T cells are involved in orchestration of the polarization of the immune response and in the expression of cytotoxicity toward pathogens and abnormal cells (Bonilla and Oettgen, 2010). The exact role of T and B cells in the pathogenesis of adenomyosis remains poorly understood. Six studies evaluated the lymphocytes present in adenomyosis eutopic or ectopic endometrium (Ota *et al.*, 1996; Bulmer *et al.*, 1998; Jones *et al.*, 1998; Yang *et al.*, 2004; Gui *et al.*, 2014; Scheerer *et al.*, 2016) (Table III).

An increase in T-cell populations (CD3+) is frequently described in the eutopic endometrium of women with adenomyosis compared to controls (Ota *et al.*, 1996; Bulmer *et al.*, 1998; Scheerer *et al.*, 2016) (Table III). In ectopic myometrium, the increased infiltration by CD3+ cells is less clear. Moreover, an increase in lymphocyte function-associated antigen and its ligand (intercellular adhesion molecule-1, which is involved in establishment of the immune synapse and the migration of immune lymphocyte cells within tissues) was observed in the eutopic and ectopic endometrium of women with adenomyosis compared to infertile women without adenomyosis, although no precise calculation of the statistical significance was performed (Ota *et al.*, 1996).

In addition to the number of cells, the activation status is also a critical part of assessing T-cell function (CD8+ or CD4+), and this has been considered in some but not all of the studies (Table III). The main function of CD8+ (cytotoxic) T cells is to monitor all the cells of the body, so as to allow elimination of those that threaten the integrity, whereas CD4+ (T helper-inducer) T cells initiate a specific immune response when activated by secretion of a cascade of cytokines. Scheerer *et al.* (2016) found an increase in CD4+ and CD8+ lymphocyte subsets in both eutopic and ectopic endometrium compared to the control group, whereas three other studies did not find clear differences between eutopic or ectopic endometrium from adenomyosis-affected women and controls (Ota *et al.*, 1996; Bulmer *et al.*, 1998; Jones *et al.*, 1998).

The cytokine profiles of CD4+ T cells dictate their classification as Th17, Treg, Th1 or Th2, among others. Tregs are potent suppressors of inflammatory immune responses. Th17 cells are a subset of pro-inflammatory T CD4+ cells. The signal that promotes Th17 differentiation inhibits Tregs differentiation: Th17 and Treg are two lymphocyte populations with opposite actions. Based on immunohistochemical staining, Gui *et al.* (2014) found a decrease in the level of forkhead box P3 (FOXP3) transcription factor characteristic of the Treg population in both the ectopic and eutopic endometrium of women with adenomyosis compared to control eutopic endometrium (Table III). Comparison of eutopic endometrium from women with adenomyosis versus endometrium from control women also revealed an increase in IL-17A+ cells (Th17) during the mid-to-late proliferative phase of the menstrual cycle (Gui *et al.*, 2014), as reflected by overexpression of the retinoic acid receptors-related orphan receptor gamma (RORγT) Th17 T-CD4+ subset marker (Gui *et al.*, 2014). These data support a role for a Th17/Tregs imbalance in the development of adenomyosis. In addition, Gui *et al.* (2014) found that the TH17-Tregs ratio in uterine tissue positively correlated with the severity of the dysmenorrhea in both diffuse and focal adenomyosis. This pro-inflammatory state acts as a pathogenetic mechanism for the occurrence of pelvic pain in women with adenomyosis. Moreover, a Th17/Tregs imbalance has been described in women with early recurrent miscarriage (Wang *et al.*, 2010a). This can be linked to the increased risk of miscarriages in women with adenomyosis (Stanekova *et al.*, 2018; Horton *et al.*, 2019).

Regarding the differentiation into Th1 and Th2 cells, this is mostly induced by IL12/interferon (IFN)γ and IL4, respectively. Little is known regarding the Th1/Th2 balance in eutopic or ectopic endometrium of women with adenomyosis. The data in one study suggest a role for Th1 cells via an increase in IFNγ expression, as shown in cultured endometrial cells derived from the eutopic and ectopic endometrium of women with adenomyosis compared to the eutopic endometrium of controls (Sotnikova *et al.*, 2002).

There is only limited data available regarding the level of B cells and their role in women with adenomyosis (Table III). B cells represent only a small percentage of the leukocyte population in the normal eutopic and ectopic endometrium of women with adenomyosis (Bulmer *et al.*, 1998). One study found a significant increase in B cell infiltration into the myometrium of women with adenomyosis compared to control myometrium (Scheerer *et al.*, 2016).

In summary, in some studies, an increase in T cells has been described in eutopic endometrium from women with adenomyosis compared to controls, with a Th17/Tregs imbalance (an increase in Th17 and a decrease in Treg subsets) suggesting a pro-inflammatory environment, that promotes pain and reproductive disorders. More extensive phenotyping of CD4+ T cells in women with adenomyosis should be part of further studies. Moreover, further studies are warranted in regard to the B cells in eutopic endometrium of women with and without adenomyosis and in ectopic lesions.

Cytokines and other markers potentially involved in immune-inflammatory pathways

There is increasing evidence in support of a role of cytokines and other factors, such as growth factors, produced in eutopic and ectopic endometrium in the pathophysiology of adenomyosis. Some of these factors are known to mainly have pro-inflammatory functions, while others have anti-inflammatory properties. A total of 31 markers were assessed in terms of their immune involvement and they are reported in Table IV.

Several cytokines that recruit monocytes and T cells to the sites of inflammation (monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2)) produced by either tissue injury or infection (Zhihong *et al.*, 2016; An *et al.*, 2017a) or that are secreted chiefly by activated macrophages (IL1 β, IL6, etc.) (Sotnikova *et al.*, 2002; Carrarelli *et al.*, 2017), were found to be differentially secreted in uteri from women with adenomyosis versus controls, thus supporting macrophage involvement in the pathogenesis of adenomyosis. IL1β and IL6 are two pro-inflammatory cytokines, and IL1β mRNA levels

Table IV Cytokines and other markers potentially involved in immune pathways.

	Adenomyosis eutopic endometrium compared to controls	Adenomyosis ectopic endometrium compared to controls	References
Pro-inflammatory factors			
MCP-1	↓		Ulukus (2005)
	↑		Zhihong (2016), An (2017a)
IL1β	↑	↓	Sotnikova (2002)
	↑	↑	Carrarelli (2016)
IL6	=		Yang (2006a,b)
	↑		Zhihong (2016)
IL6 signal transducer		↑	Chen (2010a,b)
TNFα		↑	Sotnikova (2002)
	=		Yang (2006a), Zhihong (2016)
TNFAIP6		↑	Chen (2010a,b)
IFN α	↑	↑	Sotnikova (2002)
IFN γ	↑	↑	Sotnikova (2002)
IL8	↓	↓	Sotnikova (2002)
	↓		Ulukus (2005)
	=		Yang (2006a,b), Zhihong (2016)
NFkB	↑	↑	Nie (2009)
		↑	Li (2013)
IL17a		↑	Gui (2014)
COX-2		↑	Chen (2010a,b), Li (2013)
	=°		Ota *et al.* (2001)
	=		Tokyol *et al.* (2009)
COX-1		↓	Chen (2010a,b)
Prostaglandin I2		↑	Chen (2010a,b)
Prostaglandin Receptor (F and E2)		↑	Chen (2010a,b)
Endothelial nitric oxide synthase	↑		Ota (1998)
Ucn		↑	Carrarelli (2016)
CRH	↑	↑	Carrarelli (2016)
Anti-inflammatory and regulatory factors			
IL10	↑	↑	Wang (2009)
	=		Yang (2006b)
	↓		Zhihong (2016)
TGFβ1	↑		An (2017b)
		↑	Cai (2018)
SIAE	↑		Herndon (2016)
IL33		↑	Chen (2010a,b)
IL22	↑*	↑*	Wang (2014)
IL-22R1	↑*	↑*	Wang (2014)
IL-10R2	↑*	↑*	Wang (2014)
Others factors			
NGF		↑	Carrarelli (2016)
	↑		Li (2015)
Ninj1	=*	↑*	Miyashita *et al.* (2019)
HGF	↑		Khan (2015)
EGF	↑	↓	Sotnikova (2002)
TF	↑	↑	Liu (2011)
		↑	Li (2013)

°, loss of cyclic variation; *, no calculation of significance; ↑, Significant increase compared to control endometrium or control endometrial cells; ↓, Significant decrease compared to control endometrium or control endometrial cells; =, no difference observed compared with controls.

COX, cyclo-oxygenase; CRH, corticotropin-releasing hormone; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IFN, interferon; MCP-1, monocyte chemoattractant protein 1; NFkB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGF, nerve growth factor; Ninj1, nerve injury-induced protein 1; SIAE, sialic acid acetylesterase; TF, tissue factor; *TNFAIP6*, TNF alpha-induced protein 6; Ucn, urocortin.

were increased in eutopic and ectopic endometrium of women with adenomyosis compared to controls (Carrarelli *et al.*, 2017). The IL1β cytokine level was also increased in eutopic but not in ectopic endometrial culture cells derived from the endometrium of women with adenomyosis compared to controls (Sotnikova *et al.*, 2002). In regard to IL6, which is a pleiotropic cytokine that plays an important role in immune regulation and inflammation, one study found an increase in the level of IL6 in endometrial biopsies from women with adenomyosis compared to controls, although in this report all of the patients had received controlled ovarian stimulation (Zhihong *et al.*, 2016). The IL6 signal transducer gene was significantly upregulated in ectopic adenomyosis-derived mesenchymal stem cells compared to control eutopic endometrium-derived mesenchymal stem cells: this gene codes for a signal-transducing protein shared by many cytokines, including IL6 (Chen *et al.*, 2010a). However, two studies from the same team found no significant difference in IL6 levels in eutopic endometrium from women with adenomyosis compared to controls, whether in endometrial cells cultured *in vitro* or by tissue immunostaining (Yang *et al.*, 2006a,b).

Pro-inflammatory signals in eutopic and ectopic endometrium have also been highlighted by the increased levels of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) proteins in women with adenomyosis compared to controls (Nie *et al.*, 2009; Li *et al.*, 2013). NF-*k*B is a transcription factor that controls many functions, including immune and inflammatory responses. In addition, the increase in NF-*k*B in eutopic and ectopic endometrium of women with adenomyosis correlates with the severity of the dysmenorrhea (Nie *et al.*, 2009). Moreover, the pro-inflammatory cytokine TNFα (an activator of the NF-κB pathway) contributes to the maintenance of an inflammatory activation state, specifically in the ectopic compartment (Sotnikova *et al.*, 2002). It is likely that the increased production of pro-inflammatory molecules, such as IL1β, IL6, TNFα and NF-*k*B, can induce neuronal receptor activation and cause hyperalgesia and dysmenorrhea (Li *et al.*, 2008). Nerve endings in the myometrium of women with adenomyosis can be stimulated by various inflammatory substances, including IL, growth factors (epidermal growth factor (EGF), transforming growth factor-beta (TGFβ) and nerve growth factor (NGF)), that have been reported to be differentially secreted in adenomyosis-affected uteri compared to controls (Table IV).

Although an increase in some inflammation markers is present in adenomyosis uteri, an increase in anti-inflammatory signals or factors involved in regulation of the immune response has been also reported in uterine tissues from adenomyosis patients versus controls. Studies have also shown that anti-inflammatory cytokines produced by various cell types accumulate in the microenvironment of ectopic adenomyosis lesions or eutopic endometrium (Table IV). For example, the cytokine IL8, which is a neutrophil chemotactic factor, was found to be decreased in two studies in adenomyosis samples compared to controls (Sotnikova *et al.*, 2002; Ulukus *et al.*, 2005): In an *in vitro* study, in supernatants of 24-h cultures of mononuclear cells obtained from the eutopic and the ectopic endometrium of women with adenomyosis in the proliferative phase, the concentration of IL8 was reduced compared to normal endometrium (Sotnikova *et al.*, 2002). These results were confirmed by Ulukus *et al.* (2005) *in vivo*, who found a decrease in the level of IL8 in the eutopic endometrium of women with adenomyosis compared to the endometrium of unaffected women in the secretory phase (Table IV). IL10, a cytokine with potent anti-inflammatory properties that prevents damage to the host and that maintains normal tissue homeostasis, was investigated in three studies (Yang *et al.*, 2006b; Wang *et al.*, 2009; Zhihong *et al.*, 2016). One study showed an increase in IL10 immunostaining in both eutopic and ectopic endometrial tissue in adenomyosis-affected women compared to the controls (Wang *et al.*, 2009). One study reported a decrease in the level of IL10 in the secretory phase of eutopic endometrium of women with adenomyosis in the context of ovarian hyperstimulation (Zhihong *et al.*, 2016), whereas another reported no difference compared to control endometrium (Yang *et al.*, 2006b).

Overall, these findings depict an altered local immune environment with a deregulated balance between pro-inflammatory and anti-inflammatory signals in eutopic and ectopic endometrium from adenomyosis-affected women compared to disease-free counterparts.

Immunity in relation to sex steroid hormones in adenomyosis

Sex steroid hormones have been implicated in the pathogenesis of adenomyosis (Vannuccini *et al.*, 2017), although how their interaction with the immune system can modulate immune responses remains to be fully elucidated. One of the studies of women with adenomyosis found that progesterone receptor isoform B (PR-B) and I*k*Ba (a protein known to inhibit NF-*k*B) immunoreactivity were significantly reduced in ectopic as well as eutopic endometrium compared with normal control endometrium, while the immunostaining signal for NF-*k*B subunits increased in ectopic and eutopic endometrium (Nie *et al.*, 2009). NF-*k*B participates in the synthesis of several proteins involved in the inflammatory processes. In addition, decreased PR-B and increased nuclear p65 immunoreactivity in ectopic endometrium were statistically significantly associated with the severity of the dysmenorrhea in women with adenomyosis (Nie *et al.*, 2009). It has been demonstrated that in human myometrial cells, PR-B serves an anti-inflammatory role in the uterus by antagonizing NF-*k*B activation and cyclo-oxygenase (COX)-2 expression (Hardy *et al.*, 2006). The reduced expression of PR-B, which is anti-inflammatory, in conjunction with NF-*k*B activation, may further perpetuate the inflammation and invasion capacity, and the increase in dysmenorrhea (Li *et al.*, 2013). Another study investigated the impact of increased estradiol levels and inflammation on the expression level of NGF, via separate addition of 17β-estradiol, TNFα and cobalt chloride ($CoCl_2$) to endometrial stromal cell (ESC) cultures from women with adenomyosis and control women. The authors found that 17β-estradiol promoted NGF production in the ESC cultures from women with adenomyosis but not in the controls. TNFα promoted NGF production in both groups and $CoCl_2$ inhibited NGF production in the control ESCs while it had no effect in the adenomyosis cells. Conversely, NGF promoted the proliferation and synthesis of aromatase in adenomyosis cell cultures (Li *et al.*, 2015). An indirect method to highlight the interaction between sex hormones and immunological factors in adenomyosis involves studying the effect of medical hormonal treatment on immune mediators in adenomyosis-affected women. One of the selected articles studied the effect of progesterone treatment (medroxyprogesterone acetate or danazol) on IL6, IL8 and TNFα production by ESCs derived from women with adenomyosis versus controls. After 8 days of treatment with medroxyprogesterone acetate or danazol, the cytokine responses of ESCs derived from women with or without adenomyosis were

different: the level of IL6 mRNA was significantly higher in the adenomyosis cultures than that in the controls. No difference was observed for IL-8 and TNFα mRNA levels in the samples from women with adenomyosis versus those without adenomyosis (Yang et al., 2006a). GnRH-agonist hormonal treatment was also evaluated in the context of a link between steroid hormones and immunity in adenomyosis (Khan et al., 2010). After induction of lower sex hormone levels with GnRH-agonist, the levels of CD68, a marker of macrophages and MCP-1 were significantly reduced in the eutopic endometria of women with adenomyosis compared to untreated adenomyosis patients. No difference in CD68 was observed in the ectopic endometrium (Khan et al., 2010).

These studies suggest that a local increase in estradiol and/or a decrease in progesterone, together with modulation of the immune system, likely contribute to adenomyosis lesion development and growth, although the potential mechanisms involved require further research.

Immunity and the EMT

Involvement of the EMT in the pathogenesis of adenomyosis has been described in many studies (Chen et al., 2010b; KHan et al., 2015; Qi et al., 2015). This process contributes to adenomyosis development according to the theory of myometrium invasion by endometrial cells. In the uterus, the basalis endometrium, which is closely apposed on the inner myometrium, without a basal membrane (an area called the junctional zone), may be a prime location for EMT events. The EMT includes the loss of expression or function of E-cadherin and a reduced abundance of tight junction proteins and cytokeratins, but an increase in the abundance of mesenchymal markers, such as vimentin, α-smooth muscle actin and N-cadherin. This biological process implies that epithelial cells lose their polarity and cell-to-cell contacts, to acquire a mesenchymal phenotype, which is crucial for cells to leave the epithelium and achieve the capacity to migrate (Bilyk et al., 2017; Owusu-Akyaw et al., 2019). This mechanism allows endometrial cells to migrate into the myometrium after exposure to EMT-inducing signals (Fig. 2). Several immune factors have been identified as drivers of EMT in adenomyosis (KHan et al., 2015; Qi et al., 2015; An et al., 2017a). One study reported that MCP-1 and CD68 expression, which are characteristic of macrophage activation, was higher in adenomyosis eutopic endometrium compared to controls, and that this increase correlated with the expression of N-cadherin and vimentin along with a loss of E-cadherin expression, which are hallmarks of the EMT (An et al., 2017a). In addition, the same team found that cells from adenomyosis eutopic endometrium that were co-cultured with macrophages exhibited EMT markers, suggesting that macrophages play a critical role in EMT regulation (An et al., 2017b). Following inflammatory signals, epithelial cells become more migratory, as a result of the activation of EMT (Kalluri and Weinberg, 2009).

Notch-1, another factor with a key regulatory role in the induction of EMT (Wang et al., 2010b), has been described in adenomyosis-affected women (Qi et al., 2015). Notch is known to participate in immune cell differentiation, activation and the inflammatory response (Radtke et al., 2010). Notch1 expression is increased in the ectopic endometrium of women with adenomyosis compared with normal endometrium, based on immunostaining, as are levels of the EMT-related proteins N-cadherin, Snail and Slug. Conversely, the level of Numb, which is a negative regulator of Notch signaling, was significantly reduced in adenomyosis (Qi et al., 2015). In the EMT process, Notch signaling cross-talks with various factors relevant to the EMT, such as Snail, Slug and TGF-β genes. The TGF-β signaling pathway has also been described as an activator of the EMT in adenomyosis (Cai et al., 2018). In addition to the direct effects of the Notch intracellular domain, indirect regulation of the EMT through various signaling pathways that are implicated in the physiopathology of adenomyosis, notably NF-κB, can be driven by the Notch pathway (Gonzalez and Medici, 2014). Another study reported that the tissue concentration of hepatocyte growth factor (HGF), a potent mitogen for hepatocytes and an immune regulator, was increased at the endomyometrial interface derived from focal and diffuse adenomyosis compared with control women. Interestingly, the addition of HGF to endometrial cells *in vitro* induced an increase in N-cadherin and a decrease in E-cadherin expression, thus suggesting a potential effect on EMT regulation (KHan et al., 2015).

EMT in the pathogenesis of adenomyosis can also be induced via platelet activation (Liu et al., 2016). Aside from their role in hemostasis, platelets also have immunological functions (Speth et al., 2013). Liu et al. (2016) have demonstrated increased platelet aggregation in adenomyosis lesions, as well as an increase in TGF-β1 levels, concomitant with changes in EMT markers (reduction in E-cadherin but an increase in vimentin) compared to controls. These results are in keeping with previous findings in a mouse model (Shen et al., 2016). Moreover, the levels of tissue factor (TF) and Von Willebrand factor (VWF), two proteins implicated in platelet aggregation and inflammation, are higher in the eutopic and ectopic endometrium of adenomyosis-affected women compared to controls (Liu et al., 2011; Li et al., 2013; Nie and Liu, 2016). This suggests that inflammation and platelet aggregation interact, potentially promoting EMT in the development of adenomyosis lesions.

In adenomyosis, the EMT process can be initiated in response to tissue injury, leading to local tissue inflammation and platelet aggregation. Several potential interconnected factors drive the expression and activation of EMT signals, notably activated platelets, macrophages, HGF and Notch, and possibly other immune signals (Fig. 2). Further studies are required to identify, analyze and address the underlying immune-linked mechanism implicated in the EMT in adenomyosis.

Discussion

This study provides an overview of the changes in immunological parameters that occur in women with adenomyosis. A number of immune-related markers are altered in both the eutopic and ectopic endometrium of women with adenomyosis as well as in the periphery, at a systemic level. Inflammatory as well as anti-inflammatory signals coexist in adenomyosis and therefore give rise to an immune imbalance. T cells and macrophages were the most studied immune cells. Some data suggest that T-cell populations and macrophages are increased in the eutopic endometrium of women with adenomyosis, thus supporting the notion that both innate and adaptive immunity are involved in the development of adenomyosis. Perturbation of a number of immune markers has frequently been reported in ectopic endometrium. However, the increase in T cells and macrophages is less convincing, given the heterogeneity of the available data. The findings show an imbalance between pro-inflammatory cytokines/anti-inflammatory

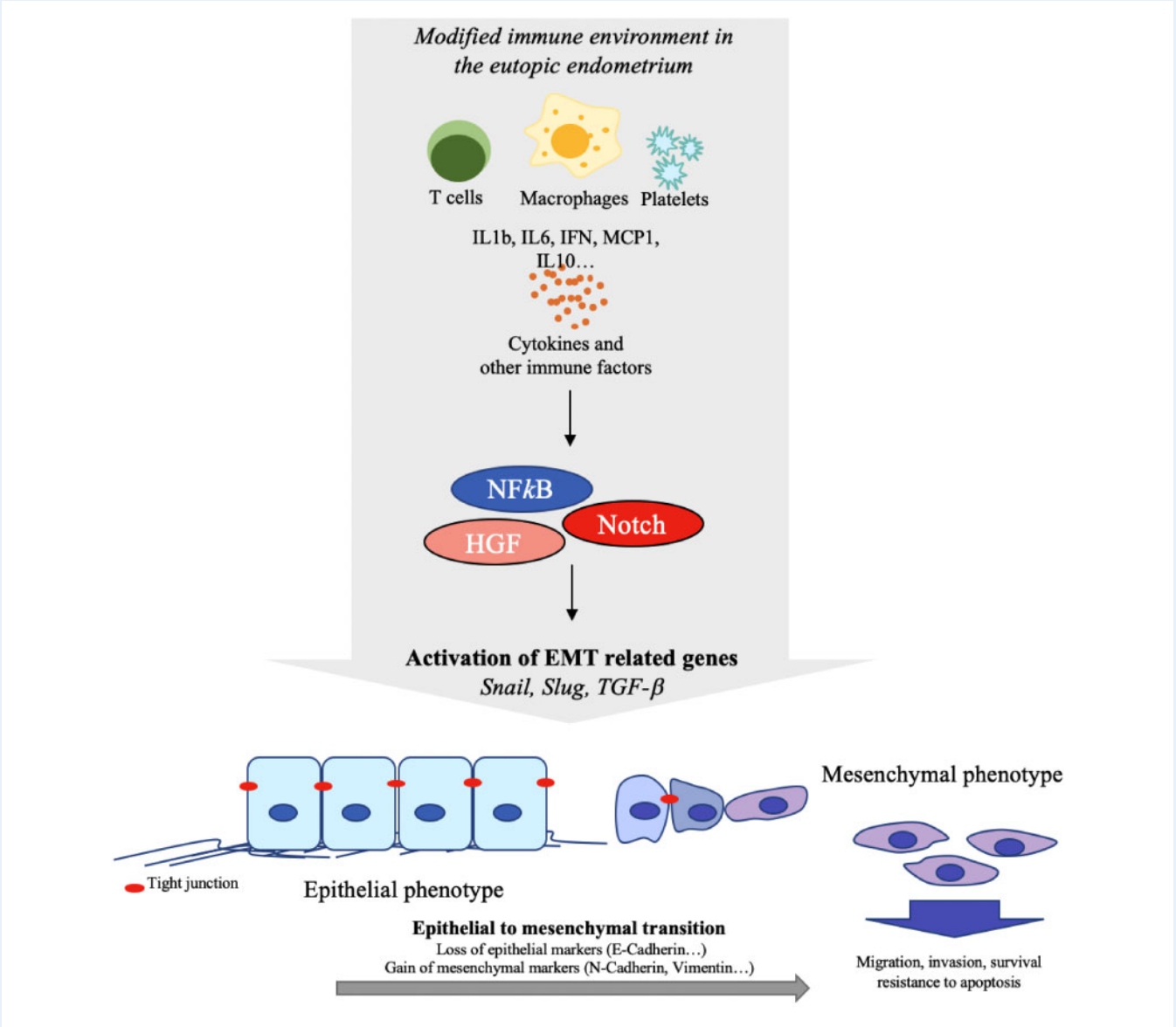

Figure 2. The hypothesis of epithelial to mesenchymal transition in adenomyosis. An epithelial to mesenchymal transition (EMT) occurs in the uterus of women with adenomyosis. This has been demonstrated as a reduction in the expression of E-cadherin (a specific marker for epithelial cells) in endometrial cells from women with adenomyosis and, in parallel, an up-regulation of mesenchymal markers such as vimentin. In adenomyosis, the immune system has been shown to be associated with the initiation of the EMT. The presence of cytokines and other immune factors secreted by macrophages, platelets and T cells in adenomyosis eutopic endometrium trigger the EMT. Epithelial cells lose a number of properties, such as cell–cell adhesion and cell polarity, and they develop properties specific to mesenchymal cells, such as migration and invasion, survival and resistance to apoptosis. HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; MCP1, monocyte chemoattractant protein 1; NFkB, NF*k*B, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF, transforming growth factor.

cytokines and other immune markers in uteri affected by adenomyosis, with an increase in the levels of a number of pro-inflammatory factors (IL1β, IL6, IFNα, IFNγ, TNFα, etc.) (Sotnikova *et al.*, 2002; Nie *et al.*, 2009; Carrarelli *et al.*, 2017; Zhihong *et al.*, 2016) and an increase in anti-inflammatory signals or factors involved in regulation of immune responses (IL10, TGFb1, IL22, etc.) (Wang *et al.*, 2009; Herndon *et al.*, 2016; An *et al.*, 2017b; Cai *et al.*, 2018). Thus, an altered local immune environment with a deregulated balance between pro-inflammatory and anti-inflammatory signals associated with platelet activation could promote the migration of endometrial cells into the myometrium in women with adenomyosis as a result of an EMT (Fig. 2). Although a direct correlation between immune disorders and sex steroids has not been studied extensively in adenomyosis, sex hormones along with local inflammation appear to be implicated in the migration of endometrial cells through the myometrium (Nie *et al.*, 2009; Khan *et al.*, 2010; Li *et al.*, 2015; Guo, 2020).

To the best of our knowledge, this systematic review is the first to examine involvement of the immune system in adenomyosis, as well as the close relationship of sex hormone effects and the EMT. We have reviewed the various immune mediators that are reported to have altered levels in eutopic and ectopic adenomyosis lesions. We have also discussed the systemic modifications in women with adenomyosis compared to unaffected women. Moreover, we have reviewed their relationship with sex steroids, which are known to have a major involvement in the pathophysiology of adenomyosis, as does the EMT. Thus, our review provides a picture of the current scientific knowledge regarding the involvement of the immune system in adenomyosis. A better understanding of the role played by the immune system in adenomyosis will lead to new research opportunities that eventually yield new treatments for the disease. A particular strength of this systematic review lies in the strict methodology based on the PRISMA guidelines. In addition, the quality of the included studies was evaluated using the Newcastle–Ottawa scale, which is a specific tool for assessing the quality of non-randomized studies in a systematic review.

Despite the precautions taken, one of the limitations of this systematic review is that it is based on observational studies with small sample sizes, most of which did not adjust for confounders, and hence the presence of bias cannot be excluded. Moreover, this review suffers from the heterogeneity of the included studies. The control women were not 'community control' women but mostly women suffering from other gynecological diseases, which can differ from one study to the next (Moritz *et al.*, 1997). It cannot be ruled out that a small number of women with endometriosis were included in the control groups. There was a degree of heterogeneity for the women with adenomyosis, due to the absence of a clear definition of the disease phenotype (focal and/or diffuse form) in some of the published studies and the fact that a variety of tools were used for the diagnosis. In addition, the association with endometriosis was not described in all of the studies on women with in adenomyosis. Another limitation of this review is the heterogeneity of the methods used to characterize the immune mediators. For example, immunochemistry is one of the most commonly used methods to identify immune populations in eutopic and ectopic endometrium. However, its quantification can be subjective, as it is often based on a small area of fixed tissue and it does not provide a direct readout of the activity of the studied cells.

The immunological changes associated with adenomyosis are complex. The findings of this systematic review are in agreement with the endometrial cell invasion theory previously described in the pathogenesis of adenomyosis. It is thought to be triggered by microtrauma in the endometrium caused by several potential intrinsic and extrinsic factors, resulting in tissue injury and hypoxia followed by repair mechanisms (Leyendecker and Wildt, 2011; Guo, 2020). One pathophysiological hypothesis states that, in response to tissue injury, an inflammatory process combined with platelet aggregation is initiated that leads to endometrial abnormalities, with potentially impaired steroid biosynthesis and increased endometrial invasive potential. A subsequent cascade of events involving an EMT can then contribute to the establishment of ectopic endometrial lesions in the myometrium (Fig. 3).

Several factors are responsible for microtrauma of the endometrium during a woman's life that causes chronic damage to the junctional zone, where the endometrium lies adjacent to the myometrium (Leyendecker and Wildt, 2011; Guo, 2020). Hyperperistalsis, induced by the local production of estrogen, would constitute a vicious cycle resulting in increased desquamation of fragments of basal endometrium (Vannuccini *et al.*, 2017; Guo, 2020). Other intrinsic factors (including the association with another inflammatory disease, such as endometriosis or possibly autoimmune diseases, exposure to a chronic endometrial infection or microbiota abnormalities) or iatrogenic trauma (endouterine procedures or particular obstetrical histories) also act as triggers. These wound injuries are characterized by an inflammatory phase. The role of inflammation in adenomyosis is supported by the afflux of macrophages, T lymphocytes, the activation of platelet and other inflammatory markers, notably the increase in NF-*k*B-binding activity induced by IL1 and TNFα, which promotes the transcription of a number of pro-inflammatory cytokines, chemokines and genes involved in proliferation, invasion, angiogenesis and oxidative stress (Sotnikova *et al.*, 2002; Nie *et al.*, 2009; Li *et al.*, 2013). A healing process, involving the activation of anti-inflammatory signals, follows wound injury in order to restore homeostasis and tissue repair. Increased levels of TGFβ or IL10 have been observed in uteri affected by adenomyosis. Therefore, inflammatory and anti-inflammatory signals coexist in the uterus of women with adenomyosis, thereby contributing to chronic perturbation of the immune environment, and may delay or prevent recovery from the injury. Estradiol and progesterone dysregulation in the uterus of women with adenomyosis acts as a master regulator of the inflammatory process (Khan *et al.*, 2010). Estrogen is also a potent stimulus of COX-2, which in turn leads to increased levels of prostaglandins in the uterus. In ectopic adenomyosis lesions, the levels of COX-2, prostaglandin I_2, prostaglandin F receptor and prostaglandin E receptor 2 are increased (Chen *et al.*, 2010a), and this can potentiate the inflammation and subsequent angiogenesis and proliferation of endometrial cells, thereby inducing a vicious cycle (Harmsen *et al.*, 2019). This altered immune environment can also induce endometrial cells to invade the myometrium through a mechanism involving an EMT (Chen *et al.*, 2010b; KHan *et al.*, 2015; Qi *et al.*, 2015) (Fig. 3).

Adenomyosis symptoms and immune changes

Pain, AUB and subfertility are the three main medical issues in women affected by adenomyosis. As well as being potentially implicated in the pathogenesis of adenomyosis lesions, the immune modifications described in these women could lead to clinical symptoms.

Although the precise mechanisms that give rise to the pain symptoms are still unknown in the specific case of adenomyosis, one accepted explanation for dysmenorrhea is the overproduction of COX-2 and prostaglandins, which can lead to abnormal myometrial hypercontractility, vasoconstriction and hypersensitization of pain fibers, thus enhancing pain symptoms (Iacovides *et al.*, 2015). In adenomyosis, an increase in NGF is observed in eutopic and ectopic cells (Li *et al.*, 2015; Carrarelli *et al.*, 2017): NGF play a critical role in neural plasticity and the release of inflammatory factors that have been described as responsible for pain (McMahon, 1996; Nie *et al.*, 2009; Li *et al.*, 2013). Moreover, other pro-inflammatory signals appear to be directly and positively correlated with the severity of the dysmenorrhea in affected women, such as NF-κB (Nie *et al.*, 2009; Li *et al.*, 2013), as well as the abundance of macrophages (Nie and Liu, 2016) and Th17/Tregs imbalance (Gui *et al.*, 2014). Additionally, the increase in platelet activation in affected women (Liu *et al.*, 2016) is also implicated in the

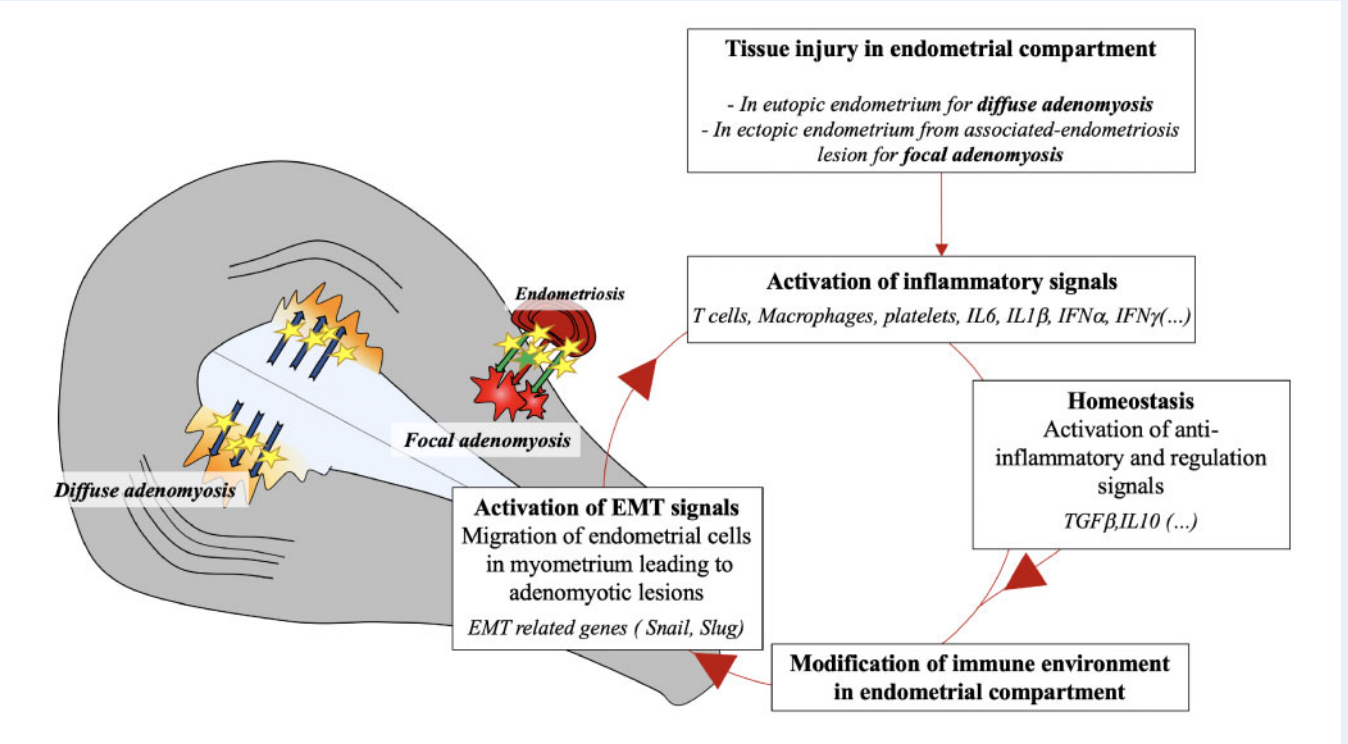

Figure 3. A hypothesis of immune system involvement in the pathophysiology of adenomyosis. A schematic representation of involvement of the immune system in the pathophysiology of adenomyosis is shown. As a result of tissue injury in the endometrial compartment (in the eutopic endometrium for the diffuse form of adenomyosis, or in the ectopic functional endometrium—from associated endometriosis—for the focal form of adenomyosis), changes occur in the immune environment of endometrial cells (represented by yellow stars). A representation of diffuse adenomyosis is indicated in orange. The dark blue arrows represent epithelial to mesenchymal cell migration 'from the inside to the outside'. The direction of the migration is from the eutopic endometrium (clear blue) to the myometrium (grey). Focal adenomyosis is indicated in red. Epithelial to mesenchymal cell migration 'from the outside to the inside' is represented by green arrows. The direction of migration is from ectopic endometrium of the endometriosis lesion (in dark red) to the myometrium (in grey).

occurrence of pain symptoms, given that anti-platelet treatment appears to be effective at improving generalized hyperalgesia and reducing uterine hyperactivity in mice (Zhu *et al.*, 2016). Therefore, in affected women, immune changes as well as platelet activation could be associated with increased uterine peristalsis, via an increase in COX-2 levels and hypersecretion of prostaglandins, which can be further causes of pain (Nie *et al.*, 2010a,b; Li *et al.*, 2013; Yang *et al.*, 2015; Guo, 2020).

These immune changes and uterine dysperistalsis in adenomyosis also have a role in impaired fertility (Kunz and Leyendecker, 2002; Kissler *et al.*, 2006). An increased frequency of uterine peristalsis during the follicular phase has been described in women with adenomyosis, which impairs the processes involved in reproduction such as directed sperm transport and fundal implantation (Kunz and Leyendecker, 2002). Moreover, several teams have described a negative impact of adenomyosis on reproductive outcomes after ART, with an increase in early pregnancy loss (Vercellini *et al.*, 2014; Younes and Tulandi, 2017; Stanekova *et al.*, 2018). The physiological immune environment necessary for a successful implantation can be altered in affected women (Tremellen and Russell, 2012; Robertson and Moldenhauer, 2014; Zhihong *et al.*, 2016) as suggested by the changes in immunes cells and cytokine secretion observed in adenomyosis endometrium compared to controls (Tremellen and Russell, 2012; Zhihong *et al.*, 2016).

Finally, adenomyosis is also associated with AUB. The ectopic endometrium in adenomyosis undergoes cyclic bleeding, therefore, it may undergo repeated tissue injury. In adenomyosis, AUB can be the consequence of the tissue injuries that have led to immune changes, angiogenesis and disruption of the local vasculature with an increased number of leaky vessels (Harmsen *et al.*, 2019). The correlation between AUB and increased levels of VWF, TF and NF-κB directly supports this theory (Liu *et al.*, 2011; Li *et al.*, 2013; Nie and Liu, 2016).

Although studies to decipher the precise relationship between immune changes and adenomyosis symptoms are lacking, available data provide ample direction for further scientific research in this regard. Moreover, the frequent association of adenomyosis with other benign gynecologic diseases can exacerbate adenomyosis-related clinical symptoms.

Involvement of the immune system according to the adenomyosis phenotype

An additional challenge with elucidation of the pathogenesis of adenomyosis is that different anatomical phenotypes of adenomyosis have

been described. Several classifications have been proposed, none of which currently have a reference value (Kishi et al., 2012; Chapron et al., 2017; Bazot and Daraï, 2018; Gordts et al., 2018). In essence, the classifications are based on two main phenotypic criteria for adenomyosis: on the one hand, alteration of 'internal' versus 'external' layers of the myometrium and, on the other hand, the 'diffuse' versus 'focal' character, defined by diffuse enlargement of the myometrium and an increase in the thickness of the junctional zone versus inhomogeneous circumscribed lesion in the myometrium, with indistinct margins separated from the junctional zone. Often, these two criteria overlap (Kishi et al., 2012; Bazot and Daraï, 2018; Lazzeri et al., 2018).

In light of the occurrence of different anatomical forms, some authors have suggested different pathogenic hypotheses. Kishi et al. (2012, 2017) have suggested endometrial invasion through the myometrium for 'internal' adenomyosis. However, external focal lesions have been proposed to involve extra-uterine invasion of ectopic endometrial cells from endometriosis lesions in the myometrium. The authors found two distinct expression patterns of fibrosis-related proteins in adenomyosis according to the type of lesions (Kishi et al., 2017). Along these lines, Khan et al. (2019) have studied the tissue patterns of glands and stromal cells in external focal lesions, internal diffuse adenomyosis and coexistent deep infiltrating endometriosis lesions. The authors found a similar pattern of glands and stromal cells for extrinsic adenomyosis and coexistent deep infiltrating endometriosis lesions, whereas the patterns of the glands and the stromal cells were similar to the endometrium in the cases with intrinsic adenomyosis (Khan et al., 2019). This hypothesis is also supported by Chapron et al. (2017), who found a correlation between focal lesions and the presence of deep infiltrating endometriosis. The authors also made the assumption of a 'from the outside to the inside invasion' theory, hypothesizing that ectopic endometrial cells migrate from posterior endometriosis nodules into the myometrium through the uterine serosa (Chapron et al., 2017; Khan et al., 2019). The hypothesis of involvement of innate and adaptive immunity in the endometrium, associated with a hormonal imbalance, leading to an EMT is applicable for both 'external' or 'focal' forms and 'internal' or 'diffuse' forms of adenomyosis (Fig. 2). One hypothesis is that diffuse and focal adenomyosis result from the same mechanisms involving tissue injury and repair (Fig. 3). Both pro-inflammatory and anti-inflammatory signals appear to be present irrespective of the forms of adenomyosis. Adenomyosis foci could originate from eutopic endometrium in diffuse adenomyosis lesions whereas, for the focal form, adenomyosis foci could originate from functional ectopic endometrium derived from extra-uterine endometriosis lesions in contact with the uterus (Kishi et al., 2012; Chapron et al., 2017; Guo, 2020). Therefore, different triggering factors (i.e. various intra-uterine triggers for diffuse forms and extra-uterine triggers, such as deep infiltrating endometriosis, for focal forms) may trigger the formation of these two different forms of adenomyosis. Of the published studies regarding systemic immune modification, and distinguishing the focal and diffuse phenotypes of adenomyosis, only a few found differences (Table II): compared to diffuse forms, the serum levels of TNFα and OPN (the latter is a factor that acts through the recruitment of monocytes/macrophages, and it mediates cytokine secretion in leucocytes) in women with focal forms were reduced (Streuli et al., 2017; Bourdon et al., 2018). However, another team found the same changes compared to controls regarding the Th17/Treg cell imbalance and an increase in IL6, IL10 and IL17 in both the focal and the diffuse forms of adenomyosis (Gui et al., 2014). Due to the lack of knowledge regarding the exact immune mechanism by which the various forms of adenomyosis occur, more research is needed in the field of immune dysfunction according to the different phenotypes of adenomyosis and their characterization.

The parallel between immune involvement in endometriosis versus adenomyosis

Endometriosis and adenomyosis are both common, estrogen-dependent, inflammatory disorders in women of reproductive age (Bulun, 2009; Chapron et al., 2019). The classical definition of endometriosis refers to the presence of endometrial-like tissue in aberrant locations, outside the uterus. Adenomyosis can occur on its own or coexist with endometriosis. A strong clinical relationship exists between these two diseases, which varies according to the phenotype (Chapron et al., 2019). It is still an open question whether adenomyosis should be considered to be a subtype of endometriosis. Indeed, adenomyosis is also defined by the presence of endometrial cells in aberrant locations outside the endometrium, albeit—unlike endometriosis—still in the uterus and in the myometrium. Because the two conditions share a number of remarkable similarities in terms of their definition, a parallel between immune involvement in endometriosis versus adenomyosis is interesting. Although the etiology of endometriosis has remained elusive, immunological dysfunction is thought to be a critical facilitator of the growth of ectopic lesions following retrograde menstruation of endometrial debris (Sampson, 1927). In adenomyosis, as in endometriosis, innate and adaptive immune system components initiate inflammation and tissue repair in response to the presence of endometrial components at an ectopic location. This then initiates immune cell infiltration (Riccio et al., 2018; Symons et al., 2018). However, the inability to cope with the persistent presence of tissue injury over time leads to an immune system overload and subsequent 'immune dysfunction' (Riccio et al., 2018). A large amount of data is available regarding involvement of the immune system in the pathogenesis of endometriosis. As is the case in adenomyosis, immune changes are observed in eutopic and ectopic endometrium in endometriosis (Izumi et al., 2018; Riccio et al., 2018; Symons et al., 2018; Vallvé-Juanico et al., 2019).

Innate immune cell populations are involved in the pathogenesis of endometriosis, particularly macrophages, neutrophils, dendritic cells and NK cells. As for adenomyosis, there is a greater abundance of macrophages in the eutopic and the ectopic endometrium of women with endometriosis versus normal controls (Hill et al., 1988; Berbic et al., 2009) and the role of macrophage M1/M2 polarization is a matter of debate (Bacci et al., 2009; Gordon and Martinez, 2010; Nie et al., 2018). In terms of NK cell involvement, the absolute number of NK cells in affected endometrium compared to normal endometrium is controversial, both for women with endometriosis and for those with adenomyosis (Yang et al., 2011; Giuliani et al., 2014). In the context of endometriosis, increased neutrophil infiltration has been observed in the peritoneal fluid of patients with endometriosis compared to that of disease-free women, with elevated concentrations of potent neutrophil chemoattractants such as IL8 (Tariverdian et al., 2009; Milewski et al., 2011). Dendritic cells are also believed to be involved in the immune imbalance in endometriosis (Fainaru et al., 2008; Stanic et al., 2014). In adenomyosis, regarding neutrophils, there is only a

limited amount of controversial data, and a decrease in IL8 levels was mostly found in adenomyosis endometrium compared to controls (Sotnikova *et al.*, 2002; Ulukus *et al.*, 2005). The involvement of dendritic cells has not been explored. However, similar to adenomyosis, increases in the levels of soluble factors, such as cytokines (IL1, 4, 6, 8, 10 and 33, TNFα, TGFβ1), growth factors (HGF, EGF), prostaglandins and reactive oxygen species, have been described in the peritoneal fluid and in lesions of patients with endometriosis (Riccio *et al.*, 2018; Zhou *et al.*, 2019). Inflammatory as well as anti-inflammatory signals coexist, leading to an immune imbalance, in the same manner as previously described in adenomyosis (Zhou *et al.*, 2019). Anti-inflammatory cytokines also have indispensable roles in the progression of endometriosis, including the promotion of survival, growth, invasion, differentiation, angiogenesis and immune escape of the endometriotic lesions (Riccio *et al.*, 2018; Symons *et al.*, 2018; Zhou *et al.*, 2019). However, the data suggest either a lower intensity of the first inflammatory response to 'tissue injury' in adenomyosis, or they reflect greater difficulty in measuring cell responses in adenomyosis foci.

The cell-mediated and humoral components of adaptive immunity that are regulated by T cells and B cells, respectively, are also implicated in the pathogenesis of endometriosis. In terms of B cells, there has been an insufficient number of studies to be able to draw conclusions regarding their involvement in adenomyosis. Conversely, there has been ample description of the involvement of B cells in endometriosis, and most of the studies to date have demonstrated an increase in the number and/or the activation of B cells, although there is still a degree of controversy in this regard (Riccio *et al.*, 2017, 2019). In terms of T-cell populations, the number of Th17 cells increases in the peritoneal fluid in endometriosis and with the disease severity (Gogacz *et al.*, 2016). Although still controversial, elevated numbers of Treg cells have been observed in the peritoneal fluid of endometriosis patients compared to controls, whereas the numbers of peripheral blood Treg cells were reduced (Berbic *et al.*, 2010; Olkowska-Truchanowicz *et al.*, 2013). Very little is known regarding Th1 and Th1 subsets in endometriosis and adenomyosis (Vallvé-Juanico *et al.*, 2019). Therefore, similar T-cell subsets are implicated in the pathogenesis of adenomyosis, although most of the T-cell modifications are observed in eutopic lesions, unlike endometriosis where most of the modifications are observed in ectopic lesions (Vallvé-Juanico *et al.*, 2019). This may be explained by the fact that 'tissue injury' initially occurs at the eutopic endometrium level in case of adenomyosis, whereas for endometriosis it occurs in the peritoneal cavity, after the displacement of endometrial tissue to ectopic locations. Perturbation of the immune environment are also associated with EMT in endometriosis, as described in adenomyosis (Grund *et al.*, 2008; Chen *et al.*, 2019; Zhang *et al.*, 2019). An EMT can lead to resistance to anoikis (thereby contributing to the survival of endometrial cells after their detachment from the extracellular matrix) and the spread of ectopic lesions, as well as an increase in lesion invasion and the formation of fibrotic tissue in and around the lesion (Young *et al.*, 2013; Yang and Yang, 2017). In addition, immune and endocrine pathways can converge to promote disease progression in endometriosis (Bukulmez *et al.*, 2008; Han *et al.*, 2015). The local biosynthesis of estradiol by endometriotic lesions in concert with pronounced inflammation within the peritoneal cavity promotes an aberrant immune-endocrine microenvironment that is ideal for growth and survival of ectopic lesions by EMT (Yang and Yang, 2017). Even though the pathogenesis and pathophysiology of endometriosis and adenomyosis are not completely understood, there is substantial evidence for a joint immunological dysfunction involving inflammation and defective homeostasis against inflammation in autologous tissue deposited in the peritoneal cavity in endometriosis, or in the myometrium in adenomyosis, thereby facilitating the growth of ectopic lesions and ultimately perpetuation of disease symptoms. Although the immune system is associated in the pathogenesis of both diseases, it is difficult to clearly understand its involvement in the development of each disease.

Conclusion

It is a challenge to compare results from different studies, but considering the issues described above, we conclude that immune pathways are clearly associated with the pathophysiology of adenomyosis. Both systemic and local immune changes have been described in women with adenomyosis. Innate and adaptive immune responses, including an increase in the number of macrophages and T cells, and in the levels of cytokines and other immune markers, result in a perturbed immunological environment in both the endometrium and myometrium, with the coexistence of inflammatory as well as anti-inflammatory signals, reflecting 'tissue injury and repair' mechanism and attempts at homeostasis. Moreover, the influx at a local level of immune cells that secrete various cytokines or growth factors intimately linked to platelet activation could be the cause or the consequence of a change in sex steroid levels and an EMT leading to the migration of epithelial cells through the myometrium. Adenomyosis symptoms, whether pain, bleeding or fertility issues, are linked to this disturbed immune environment present in the uterus of affected women. However, given the existence of various anatomical forms, interpretation of the immunological process is complex and a number of questions remain unanswered. More research is needed to better understand the pathophysiology and the early immune pathways implicated in the initiation of adenomyosis and its phenotypes.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

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Authors' roles

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Conflict of interest

The authors have no competing interests to declare.

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