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Human pregnancy: the role of chemokine networks at the fetal–maternal interface

Kristy Red-Horse, Penelope M. Drake and Susan J. Fisher

Chemokines are multifunctional molecules initially described as having a role in leukocyte trafficking and later found to participate in developmental processes such as differentiation and directed migration. Similar events occur in pregnancy during development of the fetal–maternal interface, where there is extensive leukocyte trafficking and tissue morphogenesis, and this is accompanied by abundant chemokine expression. The relationship between chemokines, leukocytes and placental development is beginning to be delineated. During pregnancy a specialised population of maternal leukocytes infiltrates the implantation site. These leukocytes are thought to sustain the delicate balance between protecting the developing embryo/fetus and tolerating its hemiallogeneic tissues. A network of chemokine expression by both fetal and maternal components in the pregnant uterus functions in establishing this leukocyte population. Intriguingly, experiments investigating immune cell recruitment revealed the additional possibility that chemokines influence aspects of placental development. Specifically, cytotrophoblasts, the effector cells of the placenta, express chemokine receptors that can bind ligands found at key locations, implicating chemokines as regulators of cytotrophoblast differentiation and migration. Thus, as in other systems, at the fetal–maternal interface chemokines might regulate multiple functions.

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To prepare for the presence of a fetus, the uterus undergoes a critical process termed decidualisation that, in mice and probably humans, is required for reproductive success (Ref. 1). This process involves phenotypic changes in uterine stromal cells that enable them to support implantation and placental development. Accompanying this transformation is the recruitment of a specialised population of maternal immune cells. These cells, termed decidual leukocytes, are a significant presence, composing up to 70% of cells in the uterus throughout early pregnancy (Ref. 2). At first glance, the recruitment of immune cells to the site of implantation seems at odds with maternal tolerance. However, the unique characteristics of decidual leukocytes, particularly with regard to their abundance and distinct composition, suggest that they participate in protecting fetal tissues from immune attack. Thus, the mechanisms that recruit decidual leukocytes to the uterine wall provide an important component to successful pregnancy.

In this review, we first introduce the anatomy of placentation and describe the composition of the placental immune-cell infiltrate. Next, we provide a detailed description of chemokine receptor and ligand expression at the fetal–maternal interface, and discuss the functional implications of the patterns found for the recruitment of decidual leukocytes. We then discuss evidence that suggests a new hypothesis: namely, that chemokines might also influence certain aspects of cytotrophoblast differentiation. Finally, we end by discussing clinical implications of chemokine-directed leukocyte recruitment and placental development in relation to pregnancy complications.

The placenta contacts the uterine wall to form the fetal–maternal interface

Placentation is the process by which fetal extraembryonic tissues establish a direct physical connection with the mother, setting up an exchange system that supports growth and development of the fetus in utero (Ref. 3). The structure of the placenta is tailored to facilitate the exchange of gases, nutrients and waste between fetal and maternal tissues (Fig. 1a). From a reductionist viewpoint, the placenta can be divided into two compartments: one that mediates fetal–maternal exchange and one that connects the fetoplacental unit to the uterus. This distinction is most dramatic in humans, where the placenta is connected to the uterus by cells derived from the extraembryonic lineage that aggressively and extensively invade the maternal uterine wall in a tumour-like process.

To form the site of fetal–maternal exchange, extraembryonic tissues develop into a disc-shaped structure composed of tree-like villi. These placental villi encase fetal blood vessels and float in the intervillous space, through which maternal blood circulates. Exchange occurs across villous surfaces from maternal to fetal blood and vice versa.

Directing maternal blood to the intervillous space is the responsibility of cells derived from the trophoblast lineage. These fetal cells, termed cytotrophoblasts, invade maternal tissues and

Figure 1. Chemokine expression at the fetal–maternal interface. *(Legend; see next page for figure.)* (a) Anatomy of the placenta.The bulk of the placenta is composed of numerous tree-like projections, called floating villi, which form the site of fetal–maternal exchange. These structures are composed of a fibroblastic core containing fetal blood vessels and a macrophage population (Hofbauer cells). This core is covered by a basement membrane and a layer of cytotrophoblast progenitors. The syncytiotrophoblast covering is formed by the fusion of the underlying cytotrophoblasts. Villi mediate the exchange of nutrients, gases and waste between fetal blood, which circulates through the stromal core, and maternal blood, which circulates through the intervillous space. The flow of maternal blood through the intervillous space is established by cytotrophoblasts that differentiate down the invasive pathway and enter the uterine wall. The invasive pathway begins when cytotrophoblast progenitors proliferate and produce a cell column that attaches to the uterine wall and forms an anchoring villus. These structures give rise to the cytotrophoblast population that invades the uterine stroma (decidua basalis) as single cells. A portion of the invading cells target uterine arterial blood vessels, which they remodel to ensure adequate delivery of maternal blood to the placenta. (b) Chemokine expression by cells located in placental villi. (c) Chemokine expression patterns by fetal and maternal cells in the uterine wall. In both (b) and (c), the location of expression of each chemokine is shown by a distinct colour, as indicated by the key on the right. For further information, see Table 1. Part (a) is reproduced (with modification) from Ref. 38, with permission from the American Chemical Society (Copyright © 2001, American Chemical Society); artwork in parts (b) and (c) is derived from part (a).

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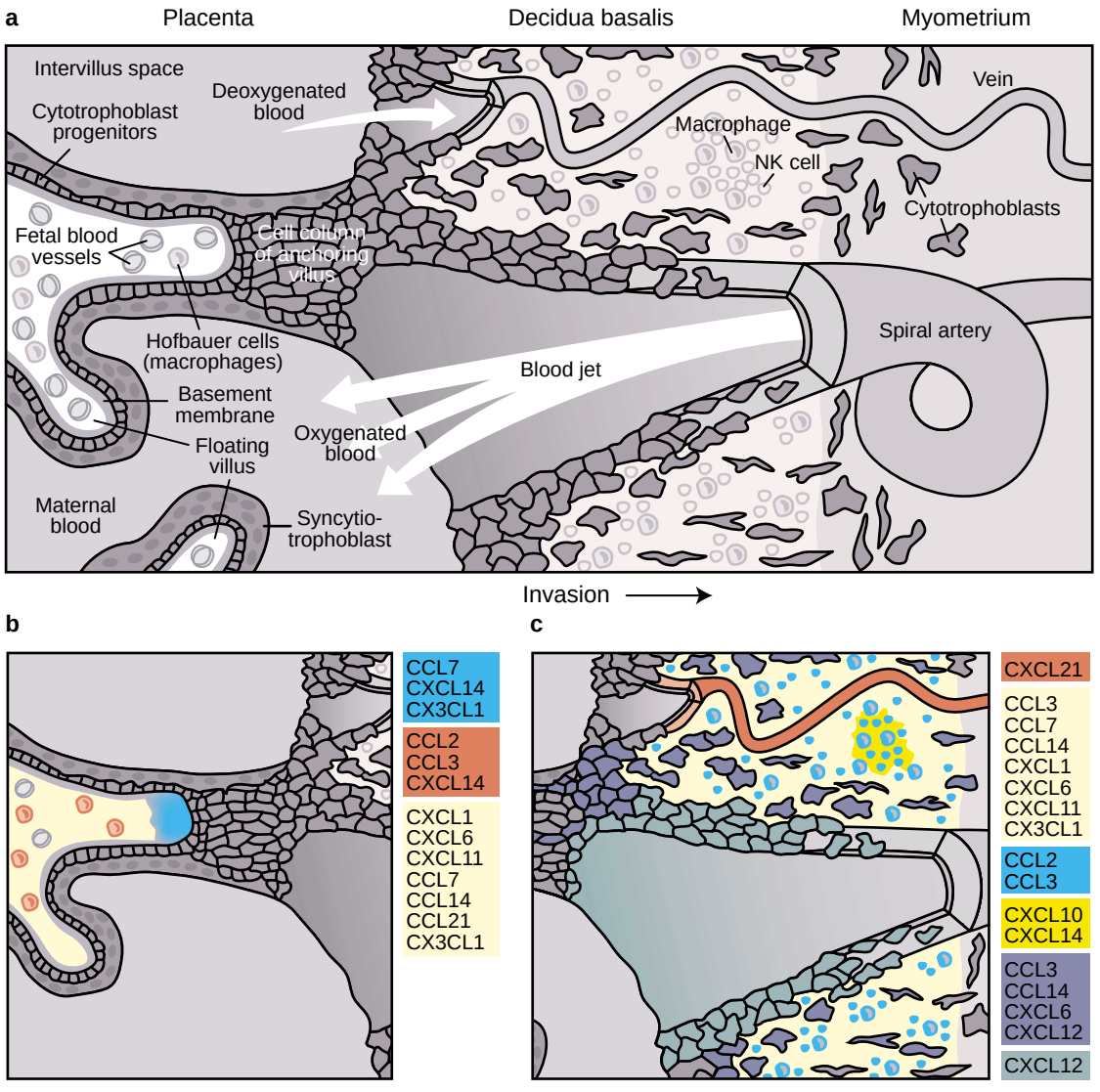

Chemokine expression at the fetal—maternal interface

Figure 1. Chemokine expression at the fetal–maternal interface. *(See previous page for legend.)*

modify the uterine wall. Here, a subset of these cells specifically migrate into the arterial circulation and replace the maternal endothelial layer and adjacent muscular wall, diverting blood flow to the intervillous space. Importantly, this temporary system also has the ability to accommodate increases in blood flow that are required in response to fetal growth.

Thus, during pregnancy, the placenta is grafted into the uterus, and fetal cells become intimately integrated into the uterine environment. From an immunological standpoint, this arrangement makes pregnancy unique. By contrast to the host response to transplantation of other organs, maternal tissues tolerate the presence of a hemiallogeneic placenta. How maternal tolerance of the embryo/fetus is ensured is not fully understood, but this enigmatic process must somehow include regulation of local immunity at the fetal–maternal interface.

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Specialised immune cells infiltrate the pregnant uterus

The decidual leukocyte population is distinct from tissue infiltrates at other locations studied. It is composed primarily (~70%) of a specialised type of natural killer (NK) cell ($CD56^{bright}CD16^{-}$), with some monocytes (~15%) and T cells (~15%), but a complete absence of B cells and granulocytes (Ref. 4). Notably, whereas $CD56^{bright}$ NK cells predominate in the pregnant uterus, they are relatively rare elsewhere, representing ~10% of the peripheral NK-cell population and less than 1% of total peripheral lymphocytes (Refs 5, 6).

Although the function of $CD56^{bright}CD16^{-}$ NK cells is only beginning to be revealed, they are known to exhibit several characteristics that distinguish them from their $CD56^{dim}CD16^{+}$ counterparts, some of which might contribute to their role in maternal tolerance. First, $CD56^{bright}$ NK cells lack the Fc receptor CD16, and therefore do not participate in antibody-dependent cellular cytotoxicity, a major NK-cell effector mechanism. Second, they are less efficient than the $CD56^{dim}$ population at killing NK-sensitive target cells (Refs 5, 7, 8). Finally, recent evidence indicates that the dominant function of $CD56^{bright}$ NK cells might be immunoregulatory. Upon stimulation, they have an increased capacity to produce regulatory cytokines such as interferon γ, tumour necrosis factor α, granulocyte–macrophage colony-stimulating factor, interleukin (IL)-10 and IL-13 (Refs 9, 10). Recent microarray analyses that compared peripheral $CD56^{bright}$ and $CD56^{dim}$ NK cells with decidual NK cells further highlight the specialisation of decidual leukocytes. Decidual NK cells differ from both these cell types in the expression of over 1000 genes, many of which have immunological functions (Ref. 11).

Taken together, these characteristics suggest a model whereby decidual $CD56^{bright}$ NK cells are a specialised population able to coexist with foreign cells and to play an important immunoregulatory role in the immunologically sensitive decidua. Thus, studying decidual leukocyte recruitment might aid understanding of maternal tolerance, and various pregnancy complications that might arise from alterations in it, as discussed further below.

Mechanisms of decidual leukocyte recruitment

The decidual environment is unique in that it must balance immunological protection of the growing embryo/fetus with tolerance of hemiallogeneic fetal tissues. It might do this, in part, by regulating immune-cell access to the implantation site – excluding potentially dangerous cells and recruiting those with protective or regulatory functions. Similar to leukocyte infiltration into other tissues, the leukocyte population in the pregnant uterus is established through the production of chemokines that attract specific immune-cell populations. Unique to pregnancy is the fact that control at the level of chemokine expression is imposed by both fetal and maternal cells (Ref. 12).

Uterine chemokines are expressed in diverse patterns that suggest specific functions

In situ hybridisation expression screens designed to characterise chemokine profiles in the decidua basalis suggest complex and overlapping regulatory functions (Ref. 12). Expression occurs in several specific patterns involving multiple cell types, including stromal fibroblasts, leukocytes, vascular endothelial cells and invasive cytotrophoblasts (Table 1; Fig. 1c). Generally, the patterns fall into four categories: (1) diffuse expression emanating from decidual stromal cells, (2) focal expression by resident leukocytes or by fibroblasts, (3) expression by invading cytotrophoblasts, or (4) localised expression in cells lining the uterine vasculature (Fig. 2). A few chemokines have more than one expression pattern.

Most of the chemokines in the pregnant uterus are in the first category. The expression of CXCL6 (GCP-2), CXCL11 (I-TAC), CCL3 (MIP-1α), CCL14 (HCC-1), CX3CL1 (Fractalkine), CXCL1 (Gro-α) and CCL7 (MCP-3) is evenly distributed throughout the decidual stroma (Fig. 2b; see Table 1 for abbreviations). However, absolute levels differ: CXCL6, CXCL11, CCL3, CCL14 and CX3CL1 are strongly expressed, whereas CXCL1 and CCL7 are found at lower levels.

CCL2 (MCP-1), CCL3, CXCL10 (IP-10) and CXCL14 (BRAK) expression patterns give focal in situ hybridisation signals. CCL2 and CCL3 are expressed in a punctate arrangement indicative of resident decidual leukocytes (Fig. 2d). CXCL14 and CXCL10 mRNAs, which are also found in foci, localise to patches of stromal cells. These patterns differ slightly: CXCL14 is found in smaller, more-numerous patches, often adjacent to clusters of decidual leukocytes, whereas

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Table 1. Chemokine expression at the fetal–maternal interface[a]

Chemokine	Uterine-wall expression				Placental-villi expression	
	Decidual stroma	Decidual leukocytes	Invasive cyto-trophoblasts	Maternal blood vessels	Villous stroma	Hofbauer cells
CXC						
CXCL1 (Gro-α)	+++				+++	
CXCL6 (GCP-2)	+++		+++		+++	
CXCL9 (Mig)	+				+	
CXCL10 (IP-10)	+++				+	
CXCL11 (I-TAC)	+++	+			+++	
CXCL12 (SDF-1)		+	+++	+++	+	
CXCL14 (BRAK)	+++					+++
CC						
CCL2 (MCP-1)	+	+++				+++
CCL3 (MIP-1α)	+++	+++	+++		+++	+++
CCL7 (MCP-3)	+++				+++	
CCL14 (HCC-1)	+++		+++		+++	
CCL21 (SLC)				+++	+	
CX3C						
CX3CL1 (Fractalkine)	+++				+++	

[a]+++, expression detected in the majority of samples tested; +, expression detected in a minority of samples tested. Data taken from Ref. 12.
Abbreviations: BRAK, breast cancer and kidney-expressed chemokine; GCP-2, granulocyte chemotactic protein 2; GRO-α, growth-related oncogene α; HCC-1, haemofiltrate CC chemokine 1; IP-10, interferon-γ-inducible protein 10; I-TAC, interferon-inducible T-cell alpha chemoattractant; MCP-1, monocyte chemoattractant protein 1; Mig, monokine induced by interferon γ; MIP-1, macrophage inflammatory protein 1; SDF-1, stromal-cell-derived factor 1; SLC, secondary lymphoid tissue chemokine.

CXCL10 expression is observed in larger stromal regions underlying these leukocyte clusters (Fig. 2f). Interestingly, CXCL14 expression is gestationally regulated: detection of the corresponding mRNA is confined to the first and second trimesters.

Several chemokines are upregulated by invasive cytotrophoblasts as they differentiate and enter the maternal tissue. Among these are CCL3 (Ref. 13), CCL14, CXCL6 and CXCL12 (SDF-1) (Refs 12, 14). Notably, the chemokines studied to date are expressed only in cytotrophoblasts differentiating down the invasive pathway; they are not seen in progenitor cells that reside in the villous core.

Finally, chemokine mRNA in the uterine wall is present in cells that line maternal blood vessels. Both CXCL12 and CXCL21 are localised to the uterine vasculature, but they are expressed in complementary patterns: CXCL12 mRNA is present in fetal cells that breach maternal arteries and replace the endothelial layer (Fig. 2h), whereas CXCL21 is expressed by maternal cells and appears to be confined to the venular endothelium.

Decidual leukocytes express receptors for chemokines found in the uterine wall

The repertoire of chemokine receptors expressed by decidual leukocytes supports the hypothesis that these cells are recruited by the complex chemokine milieu at the fetal–maternal interface (Ref. 12) (Table 2). At the mRNA level, the decidual leukocyte population as a whole highly expresses CCR1, CCR5, CXCR3, CXCR4, CX_3CR1 and the orphan receptor STRL33, whereas the receptors CCR2a, CCR2b, CCR4 and CCR7 are produced at moderate-to-low levels. Occasional expression of the receptors CXCR1, CXCR2, CXCR5 and CCR8 has been noted. In general, this observation reflects what is known about the decidual leukocyte population. For example, chemokine receptors characteristic of NK cells, T cells and monocytes are abundant (e.g. CX_3CR1,

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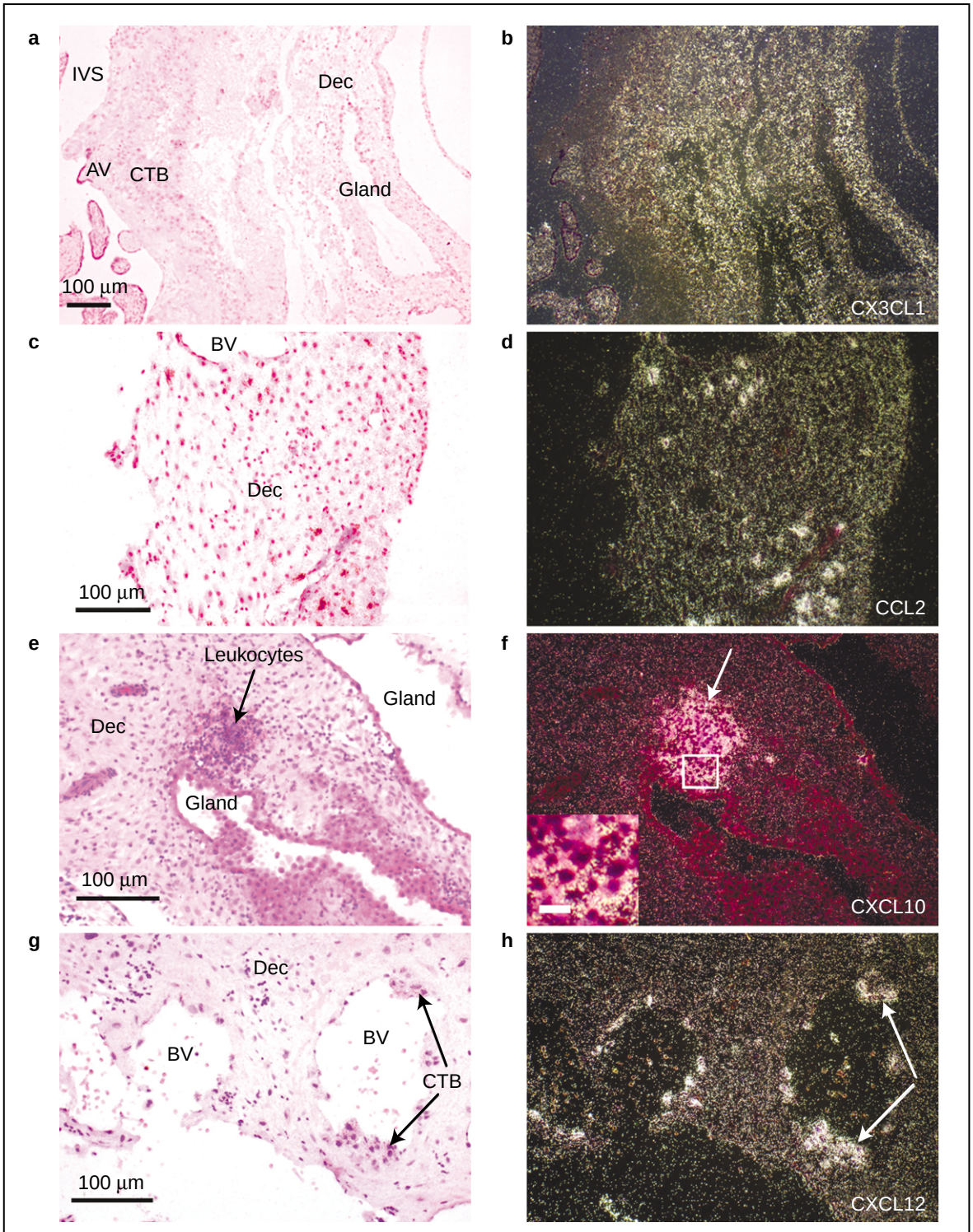

Chemokines within the uterine wall are expressed in diverse patterns

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Figure 2. Chemokines within the uterine wall are expressed in diverse patterns. *(See next page for legend.)*

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Figure 2. Chemokines within the uterine wall are expressed in diverse patterns. *(Legend; see previous page for figure.)* In situ hybridisation on tissue sections of second-trimester decidua basalis (see Fig. 1a) demonstrates the presence of chemokines in both maternal and fetal cell types. (a, c, e, g) Bright-field micrographs of histological sections stained with haematoxylin and eosin. (b, d, f, h) Dark-field micrographs of the same sections: white dots indicate signal from a ^{35}S-labelled antisense probe following hybridisation, and exposure for four weeks. (b) CX3CL1 is found in a diffuse pattern emanating from decidual fibroblasts. (d) CCL2 mRNA localises to decidual leukocytes. (f) CXCL10 is expressed in stromal cells underlying leukocyte clusters (arrow). The inset is a close-up of the area enclosed by the white box showing a cluster of CXCL10 expression, which appears to be localised to stromal cells rather than leukocytes (bar within the inset represents 10 μm). (h) Invasive cytotrophoblasts (arrows) populating maternal arteries express CXCL12. Abbreviations: AV, anchoring villi; BV, blood vessel; CTB, cytotrophoblast; Dec, decidua; FV, floating villi. IVS, intervillous; Gland, uterine gland space. Images reproduced from Ref. 12, with permission from the American Society for Investigative Pathology (Copyright © 2001, American Society for Investigative Pathology).

Table 2. Chemokine receptor expression at the fetal–maternal interface[a]

Chemokine receptor	Expression in cytotrophoblasts	Expression in decidual leukocytes
CXC		
CXCR1		+
CXCR2	+	+
CXCR3		+++
CXCR4	+++	+++
CXCR5		+
CXCR6	+++	+++
CC		
CCR1		+++
CCR2	+	+++
CCR3	+	
CCR4		+
CCR5	+++	+++
CCR7	+++	+++
CCR8		+
CX_3CR1		
CX_3CR1	+	+++
Orphan		
GPR1	+++	+

[a]+++, expression detected in the majority of samples tested; +, expression detected in a minority of samples tested. Data from Ref. 33.

CXCR3, CCR1, CCR2, CCR5 and CCR7), as are their ligands within the decidua (CX3CL1, CXCL10, CCL2, CCL3, CCL14 and CCL21).

Specific analysis of NK-cell subsets from peripheral blood shows that $CD56^{bright}$ NK cells have a chemokine receptor repertoire distinct from that of $CD56^{dim}$ cells. $CD56^{bright}$ NK cells express high levels of CCR5, CCR7, CXCR3 and CXCR4, which translates functionally to preferential chemoattraction by their ligands (Refs 14, 15, 16). Accordingly, these ligands (CCL3, CCL21, CXCL10 and CXCL12) are expressed in the decidua.

The highly suggestive distribution patterns of receptors and their cognate ligands strongly

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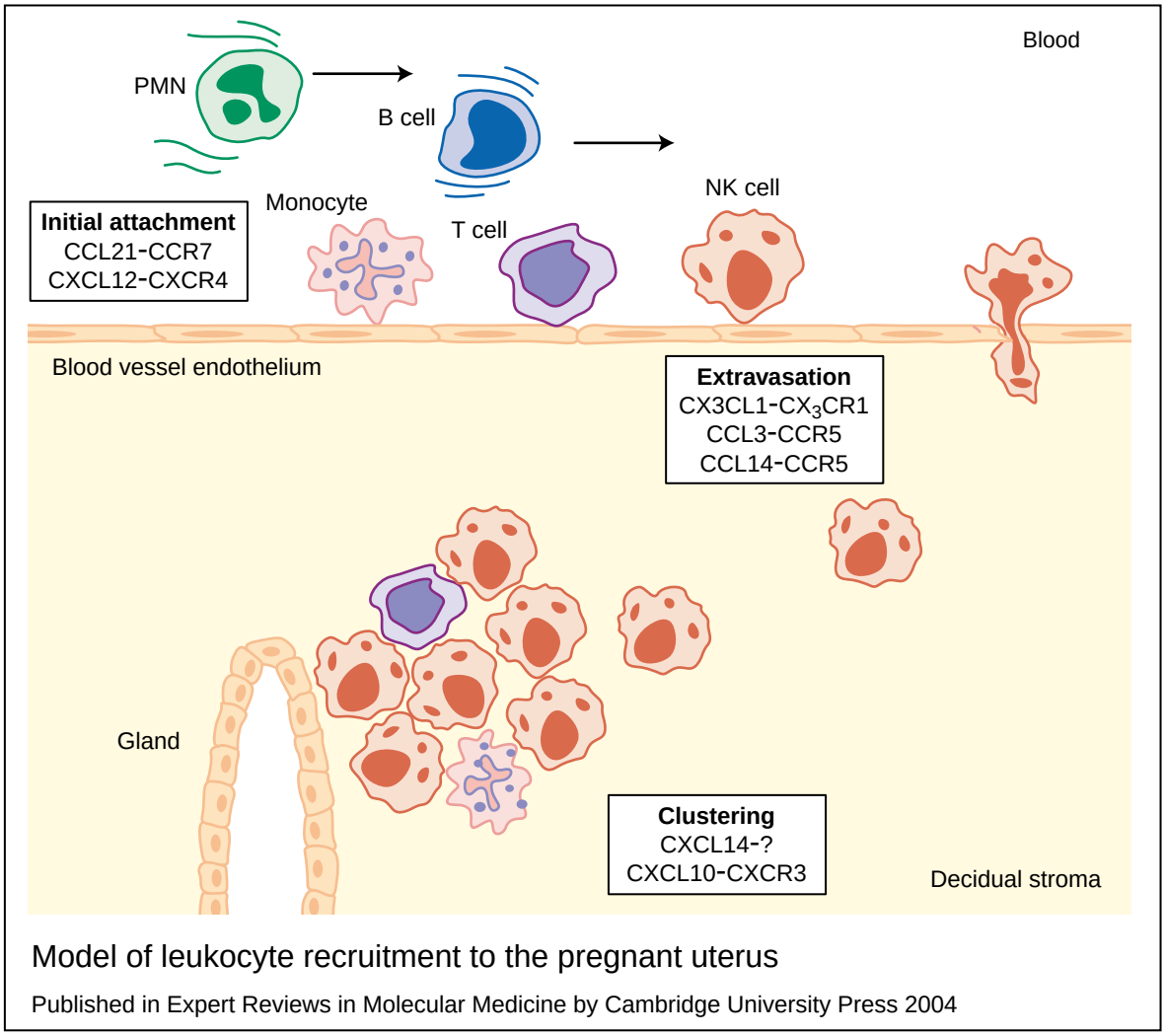
support a model whereby multiple specific interactions orchestrate the movement of particular leukocyte populations from circulating blood into the uterus (Fig. 3). The mechanisms of leukocyte trafficking under homeostatic and inflammatory conditions entail a similar stepwise cascade where adhesion molecules and chemokines combine to define the specificity of extravasation (Ref. 17). In the uterus, CD56^{bright} NK cells, T cells and monocytes destined for the decidua and expressing CXCR4 and CCR7 might encounter their ligands – CXCL12 and CXCL21, respectively – on endothelial surfaces as they circulate through uterine blood vessels. It is



Model of leukocyte recruitment to the pregnant uterus

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Figure 3. Model of leukocyte recruitment to the pregnant uterus. The model is based on ligand mRNA patterns, leukocyte receptor expression, and function of placenta-derived chemokines as monitored by in vitro chemotaxis assays. Circulating leukocytes that pass through the uterus contact CXCL12, produced by cytotrophoblasts that line maternal arterioles, and CCL21, produced by maternal cells that line uterine veins. These chemokines might trigger the attachment of CD56^{bright} natural killer (NK) cells, T cells and monocytes that express the receptors CCR7 and CXCR4. Extravasation of attached cells might be directed by interactions between the receptors CX_3CR1 and CCR5 and their ligands CX3CL1, CCL3 and CCL14 expressed in the decidual stroma. Cells could then be induced to cluster near glandular epithelium by CXCL10 and the orphan chemokine CXCL14, acting via CXCR3 and an unidentified receptor, respectively. Abbreviation: PMN, polymorphonuclear cell. Diagram based on figure from Ref. 39, with permission from Kluwer Academic/Plenum Publishers (Copyright © 2002, Kluwer Academic Publishers).

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interesting to note that these two chemokines are most selective among NK-cell populations – both have been reported to attract the CD56^{bright} subset preferentially – supporting the notion that this interaction could serve as an initial trigger for attachment (Refs 14, 16). Following initial capture, extravasation could be prompted by multiple interactions, including those between the receptor CCR5 and its ligands CCL3 and CCL14, which are found throughout the decidual stroma. Finally, once in the tissue, leukocytes might be further organised into their characteristic clusters via CXCL10–CXCR3 interactions or interactions between CXCL14 and its unidentified receptor.

Several steps in this process have been functionally assessed using in vitro models of human decidual leukocyte recruitment (discussed below). Additionally, these events are beginning to be addressed in vivo using mice. However, caution must be taken when superimposing functions at the fetal–maternal interface of the two species. The similarities make the data extremely interesting, but anatomical differences between mice and humans, which are probably mirrored at the molecular level, must be anticipated.

Experiments in mice have answered the long-standing question of whether the appearance of decidual leukocytes is regulated at the level of trafficking or by proliferation of resident cells. When uterine grafts from donors with normal NK-cell populations are transplanted into either wild-type or NK-cell-deficient mice, only those transferred into wild-type recipients contain decidual NK cells during pregnancy (Ref. 18). Thus, this cell population must originate from sites outside the uterus. Further transfer studies established that secondary lymphoid tissues are the source of the NK cells recruited to the decidua. Adoptive transfer of cells from wild-type spleens or peripheral lymph nodes from pregnant mice into NK-cell-deficient mice reconstitutes the decidual NK-cell population. The role of chemokines in this trafficking event has also been considered. CXCL1 (KC) and CCL2 (MCP-1) expression levels in the murine pregnant uterus fluctuate in sync with macrophage infiltration (Ref. 19). Pregnant mice deficient in CCR2, CCR5 or the ligand CCL3 have normal decidual leukocyte populations, indicating a nonessential function for this chemokine in mice, which may or may not be the case in humans (Ref. 20). However, given the abundance of chemokine expression at this location, it is likely that compensatory mechanisms exist.

Invasive cytotrophoblasts influence human decidual leukocyte recruitment

As fetal cytotrophoblasts migrate deeply into the uterine wall during human pregnancy, it is not surprising that they influence the maternal immunological environment. As described above, in situ hybridisation reveals that invasive human cytotrophoblasts express a multitude of chemokines. To date, experimental evidence has shown the functional relevance of two of these ligands.

To test the role of cytotrophoblast chemokine production in leukocyte migration, cytotrophoblast-conditioned medium was used in chemotaxis assays to characterise the effects of placental chemokines on peripheral blood mononuclear cells (Ref. 13). Cytotrophoblasts differentiating down the invasive pathway during in vitro culture express chemokines as they do in vivo (Refs 12, 13). In addition, chemoattractants within cytotrophoblast-conditioned medium direct the migration of leukocyte populations that are similar to those found at the fetal–maternal interface – i.e. NK cells, monocytes and T cells. CCL3 and CXCL12 are important participants in this activity. CCL3, which is involved in attracting NK cells and monocytes, is responsible for nearly 70% of the migration of CD56^{bright} NK cells and 50% of monocytes in response to stimulation by cytotrophoblast-conditioned medium (Ref. 13). CXCL12 is important in specifically attracting the CD56^{bright} population. This chemokine is produced by endovascular cytotrophoblasts, and recombinant human CXCL12 induces the migration of CD56^{bright}CD16^{-} NK cells over that of CD56^{bright}CD16^{+} NK cells (Ref. 14).

Through chemokines, cytotrophoblasts could potentially manipulate maternal immunity at multiple levels. In addition to participating in their recruitment, placental cells are equipped to regulate decidual leukocyte movement or activation state within the decidual microenvironment. This suggests a regulatory mechanism whereby cytotrophoblasts recognise and respond to changes in their immediate locale. Work in mice demonstrates such a function: in response to the presence of *Listeria monocytogenes*, cytotrophoblasts produce CXCL1 and CCL2, which function by attracting monocytes that are required to resolve the infection (Ref. 21).

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Chemokines as regulators of placental development

In addition to their long-recognised function of directing leukocyte movement, chemokines also participate in many developmental processes, including those that rely on proliferation, differentiation and targeted migration of nonhaematopoietic cell populations. With regard to mitosis, chemokines commonly stimulate proliferation and protect against apoptosis in targets such as endothelial and neuronal cells, functions that are well defined in the haematopoietic lineage (Refs 22, 23, 24). As to cell fate, the receptor CXCR4 and its ligand CXCL12 are expressed in complementary patterns in the developing neuronal, cardiovascular and haematopoietic systems, as well as in the craniofacial region of the mouse. Null mutations in either gene yield defects in these areas (Refs 25, 26). Other chemokine receptor–ligand pairs play a role in murine T-cell differentiation by influencing the balance of T helper 1 (Th1) versus Th2 cells (Ref. 27). Chemokines also function in nonimmune-cell trafficking, such as cancer cell metastasis to selected sites (Refs 28, 29) and migration of germ cell and neuronal progenitors in the developing embryo (Refs 30, 31, 32).

Organogenesis of the placenta relies on the same developmental processes and, therefore, chemokines at the fetal–maternal interface might participate in similar nonimmune functions. In support of this hypothesis, cytotrophoblasts express a broad repertoire of receptors capable of binding chemokines expressed in the decidual environment and in placental villi, a location that also contains an abundance of chemokine mRNAs (Ref. 33). Analysis of the expression of reciprocal receptor–ligand pairs indicates that chemokines have the capacity to influence cytotrophoblast differentiation and migration as the progenitors move from the trophoblast basement membrane that surrounds the villous stroma into the interstitial and endovascular compartments of the uterus. In the goat, chemokines might be involved even earlier; trophoblast migration and attachment are stimulated by CXCL10 and its localisation to receptive endometrium suggests that this ligand stimulates blastocyst attachment (Ref. 34). It is not known whether a similar mechanism functions in humans, but chemokines are cyclically regulated in the human endometrium and human blastocysts express chemokine receptors (Refs 35, 36).

Cytotrophoblasts express multiple chemokine receptors

The first indication that leukocytes are not the sole responders to chemokines at the fetal–maternal interface is that cytotrophoblasts express multiple receptors that bind a spectrum of CC, CXC and CX3C chemokines (Table 2) (Ref. 33). RNase protection assays using RNA from isolated cytotrophoblasts demonstrate that these cells consistently express relatively high levels of CCR7, CXCR4 and CXCR6 and intermediate amounts of CCR5 and GPR1. Lower levels of CXCR2, CCR2, CCR3 and CX_3CR1 are observed in some preparations. In addition, invasive cytotrophoblasts located in the uterine wall stain with an antibody specific for the receptor CCR1 (Ref. 37). By contrast to the case for cytotrophoblasts, chemokine receptor expression by placental fibroblasts, the stromal cells of the fetal compartment, is not detected. This observation most likely reflects the fact that cytotrophoblasts are a more dynamic cell type participating in specialised differentiation pathways and extensive migration.

Expression of chemokines in the placenta

Like the uterine wall, cells within placental villi are rich in chemokine mRNA. To date, the expression of 13 chemokines has been localised to this region, predominantly in two resident cell types: fibroblasts and Hofbauer cells (placental macrophages) (Table 1; Fig. 1b). With regard to specific patterns, the following chemokines are uniformly expressed throughout the stromal compartment: CCL7, CCL14, CXCL1, CXCL6, CXCL11 and CX3CL1. The chemokine CCL21 shares this pattern but is seen less consistently, with reduced signal intensity. CXCL14, CCL2, and CCL3 are expressed in a punctate manner, often clustered near the basement membrane that encases the stroma, a pattern that matches the distribution of Hofbauer cells. As in the decidua, CXCL14 is gestationally regulated such that it is present in first- and second-trimester samples and is very low or absent at term.

Perhaps the best evidence to date for chemokine function in cytotrophoblast differentiation is a novel in situ hybridisation pattern in which mRNA signals are sometimes brightest at sites underlying cell column initiation. For the chemokines CX3CL1 and CCL7, which appear to be produced by stromal fibroblasts, this pattern is characterised by a relatively broad band of

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expression that peaks in intensity near the base of cell columns. For the chemokine CXCL14, which is expressed by macrophages, narrower patches of intense expression are seen in the same region. The unusual nature of these patterns, which are confined to regions where cytotrophoblast fate is determined, suggests that this observation has potential functional importance.

Expression patterns suggest sites of cytotrophoblast–chemokine interactions

Cytotrophoblast progenitors in floating villi become the specialised effector cells that carry out many of the essential functions of the placenta. Accordingly, cytotrophoblasts must undergo a complex differentiation program that, when executed properly, results in a fully functional organ. Although immensely important to successful reproduction, the signals that regulate cytotrophoblast differentiation and morphogenesis are not well understood. Evidence of chemokines at the fetal–maternal interface suggests that these molecules might be involved. On the basis of published reports that chemokines can regulate organogenesis and influence cell fate, one can identify pairs of chemokine receptors and their ligands that might regulate aspects of cytotrophoblast differentiation (Refs 25, 26, 27).

In the fetal compartment, cytotrophoblast progenitors express several receptors that bind chemokines produced by cells that populate the stroma. Here, receptor–ligand pairs such as CCR5–CCL3, CCR5–CCL14, CXCR2–CXCL1, CXCR2–CXCL6, and CX_3CR1–CX3CL1 could be involved in maintenance of the progenitor cell population by enhancing proliferation and preventing apoptosis. Furthermore, provocative receptor–ligand expression patterns at sites of column initiation suggest that chemokines could regulate recruitment of cytotrophoblast progenitors to the invasive pathway. Specifically, CXCL6, CCL7, CX3CL1 and the orphan ligand CXCL14 signals are enhanced at a subset of these sites. In the same location, cytotrophoblasts express the known receptors for these molecules: CXCR2 (for CXCL6), CCR2 (for CCL7) and CX_3CR1 (for CX3CL1). Thus, with signals found in the underlying stroma, these chemokines are ideally positioned to direct cytotrophoblasts at the base of columns to adopt an invasive, rather than a syncytial, phenotype.

In the maternal compartment, cytotrophoblasts that migrate into the uterine wall secure attachment of the fetal unit and bring maternal blood to the intervillous space. To achieve the latter function, invading cytotrophoblasts navigate a dense decidual interstitium containing numerous maternal leukocytes before they reach uterine spiral arterioles. In this location, fetal cells commence replacing the maternal endothelial lining and disrupting the underlying muscular wall. Chemokines and their receptors could regulate several aspects of cytotrophoblast invasion/differentiation as well as survival. As invasion ensues, cytotrophoblasts express CCL3 and CCL14 as well as their receptors CCR1 and CCR5 (Refs 12, 13), suggesting an autocrine mechanism whereby cytotrophoblasts might regulate their own fate. These chemokines are also expressed diffusely throughout the decidualised uterine stroma, which implies that paracrine signals might be acting in concert to attract invading cells to the deeper portions of the uterine wall. In fact, cytotrophoblast invasion is enhanced when migrating towards media supplemented with CCR1/CCR5 ligands (Ref. 37). One of the most intriguing implications stems from the observation that invading cells that occupy maternal arterioles produce CXCL12 (Refs 12, 14), whereas the equivalent in vitro population expresses the cognate receptor CXCR4. The low level at which chemokine receptor mRNAs are expressed in vivo makes detection of these molecules by in situ hybridisation difficult. Nevertheless, the interesting possibility exists that cytotrophoblasts might use CXCL12 as a targeting signal that becomes amplified as cells populate the maternal vasculature.

Clinical implications and future directions

Pregnancy is one of the least-studied and, consequently, least-understood areas of medical science. This fact is due in part to the profound differences in placentation among even closely related species. Understanding the molecular underpinnings of normal placentation is important because disruptions in this process are associated with pregnancy complications. One of the most significant of these complications is pre-eclampsia, which affects up to 7% of all human pregnancies and is a leading cause of maternal mortality and fetal morbidity in the developing world. Particularly disconcerting is the fact that the cause of pre-eclampsia is unknown and the only effective treatment is

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delivery of the placenta. Further exploration of the molecular mechanisms orchestrating normal placental development and how they go awry is needed to stimulate the development of diagnostic tools or clinical treatments for pregnancy complications.

Chemokine functions, which regulate homeostatic, inflammatory and differentiation processes, have the potential to lie at the tipping point between normal and pathological pregnancy. Injury and infection stimulate the production of chemokines, which could disrupt normal balances in the uterus. In light of the data reviewed above, altered chemokine profiles might disrupt the normal regulation of decidual leukocyte and placental cytotrophoblast functions, contributing to multiple pregnancy complications. Future studies will be aimed at investigating the interesting possibility that modifications in chemokine expression correlate with pregnancy complications, especially pre-eclampsia and cases of preterm delivery, which are often associated with excessive inflammation. In support of this theory, we observed individual variations in chemokine expression patterns in samples with obvious cellular abnormalities such as leukocyte infiltrates (Ref. 33).

In conclusion, chemokine function at the fetal–maternal interface contributes to the regulation of the decidual leukocyte population and is therefore instrumental in governing local maternal immunity. Additionally, detailed analyses of in situ hybridisation patterns reveal provocative, reciprocal ligand and receptor mRNA expression patterns that form the basis for numerous testable hypotheses. Although, at this stage, the function of these ligands and receptors is largely unknown, their expression patterns lay a solid framework for future studies of normal and pathological pregnancies.

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Further reading, resources and contacts

A chemokine database of expressed sequence tag clones can be found at:

http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html

Features associated with this article

Figures

Figure 1. Chemokine expression at the fetal–maternal interface.
Figure 2. Chemokines within the uterine wall are expressed in diverse patterns.
Figure 3. Model of leukocyte recruitment to the pregnant uterus.

Tables

Table 1. Chemokine expression at the fetal–maternal interface.
Table 2. Chemokine receptor expression at the fetal–maternal interface.

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