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Localization of pro-inflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines at the foetomaternal interface during murine pregnancy

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SUMMARY

The involvement of some interleukins (ILs) in early and established pregnancy has been convincingly demonstrated, but little is known about the potential role of the more recently discovered ones. However, since many of these have positive or negative regulatory effects on both NK and T cells, it is highly probable that they also have regulatory functions in both implantation and placental development. Therefore, as a first step in tackling this problem, we have investigated whether several recently described pro- (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines were expressed at the uteroplacental interface by use of immunohistochemistry at different stages of gestation in mice.

Each of these molecules was found at the foetomaternal interface, with specific distributions and patterns of expression depending on both the cytokine itself and the stage of pregnancy. The significance of these data is discussed.

Keywords interleukins pregnancy Th1-Th2 decidua placenta

INTRODUCTION

The immunological success of pregnancy depends upon the establishment of a balance between immunostimulation (immunotrophism) and immunosuppression [1], the current paradigm viewing pregnancy as a Th2 cytokine-dependent phenomenon [2,3]. Deregulation of the Th1/Th2 balance leads to abortion in mice, which is prevented by r-IL10 [4–6]. An equivalent breakdown in equilibrium is seen in many cases of human infertility [7]. Although the involvement of several other cytokines, such as IL-1 [8], IL-2 [9], LIF [10], has already been studied in detail, there are still relatively few data for the more recently discovered ones, such as IL-11, IL-12, IL-13 and IL-15. Moreover, most of the available studies deal with the human situation.

IL-11, a member of the gp130 cytokine family [11] as is LIF, appears as essential as the latter for mouse embryo implantation [12]. This could be related either to its anti-inflammatory properties [13] (to the extent that it is often referred to as a member of the Th2 cytokine category), or to some of its many other biological functions, including growth-factor-like properties [14].

IL-13, predominantly secreted by activated Th2 cells, is also considered as an anti-inflammatory 'IL-4-like' molecule [15]. Mononuclear lymphocytes, B cells, large granular lymphocytes and endothelial cells, known to be responsive to IL-13, can be traced in either the placenta or the maternal reproductive tract [16,17].

In contrast to such interleukins, IL-12 is often compared to Th1 cytokines [18]. It increases IFN-γ levels, T cell cytotoxicity and NK cell activation [19]. Indeed, in mice, it triggers abortion when coinjected with IL-18 [20].

IL-15, produced by a wide variety of cells and tissues, but not by T cells, displays many IL-2-like effects, and it has been suggested as a substitute for IL-2. Its capacity to up-regulate T and NK cell differentiation and proliferation may be relevant to its action on granulated metrial gland cells in the pregnant uterus [21]. Another property, besides the anabolic ones [22], may be relevant to reproduction: the influence on the arrangement of adhesion molecules [23], which itself may be involved in the regulation of the invasion of cytotrophoblasts (demonstrated in humans with an *in vitro* model using the JEG-3 choriocarcinoma cells [24]).

Although some of these cytokines have already been found in the murine reproductive tract, data about their expression, and even more their functions during pregnancy, are rather scarce. As a first step in this study, we decided to investigate their expression in the female reproductive tract in mice by immunohistochemistry (IHC) on adjacent sections obtained at the implantation and peri-implantation periods, early gestation (day 8·5), followed by the mid (day 10·5) and late (day 19) stages of gestation.

We show here that the decidual and placental structures expressed each cytokine in a stage-dependent fashion, which

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suggests a potential involvement in cytokine interplay during pregnancy, which is discussed further.

MATERIALS AND METHODS

Mice

CBA/J and BALB/c mice were obtained from Iffa Credo (l'Arbresles, France) at the age of 8 weeks. Matings were performed either in syngenic (i.e. BALB/c × BALB/c) or nonabortion-prone allogenic combinations (CBA/J × BALB/c) leading to a genetic disparity of the MHC regions. Mice were bred under conventional conditions.

Preparation of decidual uterine tissues and placentas

Mice were mated in separate cages as 'trios' (one male, two females). The males were removed after overnight caging, dated as day 0·5. The pregnant animals were killed by cervical dislocation on days 8·5, 10·5 and 19 of gestation.

The uterine horns were dissected and isolated, and then each uterine chamber was treated individually. It was opened longitudinally, and the foeto-placental unit separated from the uterine implantation sites. Of the uterine tissues, we either kept the whole chamber or individual deciduae were peeled off from the respective implantation sites. The placentas were separated from the embryo and annexes. In a few cases, especially at day 8·5, we kept complete uterine chambers for examination of a complete site whenever possible.

The placentas and deciduae from the implantation sites or whole uterine chambers were immediately incorporated in an embedding medium (Tissue-Tek OCT Compound, Miles Inc., via Tebu, Le Perray, France; Ref. 4583), immediately snap frozen and stored at − 80°C for later use. Tissue sections (5–8 μm) were then cut in a cryostat at − 22°C and collected on slides (Super Frost Plus, Menzer Glaser, via Polylabo, Paris, France; Ref. 041300). They were then dried for 2 h at room temperature before being stored at − 80°C for later analysis by immunohistochemistry.

Immunohistochemistry

The primary labelling was performed using polyclonal goat antimouse cytokine antisera which were, respectively, anti-IL-11 (Anti-mouse IL-11 Neutralizing Antibody, Ref. AF-418-NA, R and D Systems, UK), anti-IL-12 (Anti-mouse IL-12 Neutralizing Antibody, Ref. AF-419-NA, R and D Systems), anti-IL-13 (Anti-mouse IL-13 Neutralizing Antibody, Ref. AF-413-NA, R and D Systems), anti-IL-15 (anti-IL-15 Goat Affinity Purified, Santa Cruz Biotechnology Inc., via Tebu; Ref. SC-1296). Antibodies of the same origin were used as negative controls to prove the absence of nonspecific binding (Normal Goat IgG, Ref. AB-108-C, R and D Systems). The secondary antibody was a biotinylated rabbit antigoat IgG (Ref. E466, Dako). Use was then made of the Vectastain Elite ABC Kit (Goat IgG, Ref. PK-6105, Vector Laboratories, Burlington, USA), and revelation was via addition of DAB tetrahydrochloride (DAB Substrate Kit for Peroxydase, Ref. SK-4100, Vector Laboratories).

After 15 min at room temperature, the sections were fixed in 2% PBS-formaldehyde solution at 4°C in the dark for 10 min. The sections were washed three times in 0·1% (w/v) PBS-saponin (saponin Sigma, Ref. S-2149), except for those treated with IL-15 antibody, for which all washes were in PBS. Endogenous peroxidase was blocked by incubation in the dark with a Peroxydase Blocking Solution (Ref. S-2023, Dako), and three washes of 5 min duration were again performed. As an alternative, we used the commercial Avidin/Biotin Blocking Kit (Ref. SP-2001, Vector Laboratories) as per the manufacturer's leaflet. Slides were then incubated for 45 min with 1/100 (1 mg/ml) mouse IgG solution in normal rabbit serum. The primary antibody was diluted in PBS-saponin solution at 5 μg/ml (IL-11, IL-12 and IL-13) or in PBS solution, at 5 μg/ml (IL-15). Incubation was overnight and, after three washes, the secondary antibody (1/400 in PBS-saponin solution) was added and left to react for 45 min. Three washes were performed before the final revelation with the Vectastain Kit (30 min incubation) and washes in PBS. DAB was left to react for 8 min before washing in distilled water and counter-staining by Mayer's haematoxyllin solution, and final mounting in Glycergel® (Dako, France). For lymphocyte labelling in the double staining experiments, we used a biotinylated goat polyclonal antimouse-CD2 (CD2 M16 ref sc-6989, Santa Cruz Biotechnology Inc, Tebu, France).

General procedure

Each experiment (i.e. for every mating and, for the matings themselves, the gestational stages mentioned in Results) was repeated at least twice, usually three times. There was a minimum of three different uterine chambers from the different mice studied in each case. The same organs were used in parallel for the four cytokines.

RESULTS

The overall results are assembled in Table 1, but there follows here a brief description, illustrated by some representative photographs. The IHC negative controls are shown in Fig. 1a–e.

Interleukin-11

Uterine chamber (day 8·5). IL-11 was strongly predominant in the decidualized uterine stroma and present in a homogeneous fashion around the implantation site (Fig. 2a). The inner cell mass appeared negative. Both ectoplacental cone and giant cells were slightly stained. (Fig. 2b).

Decidua. At mid-gestation, Fig. 2d showed an anti-IL-11 staining in the metrial glands and the uterine epithelium. The inner decidua appeared positive, but in a weaker and less homogeneous way (Fig. 2c).

At the end of gestation, the pattern appeared similar but weaker than earlier (Fig. 2e), with a reduction in staining of the glandular epithelium in the inner decidua (Fig. 2f).

Placenta. Only a few weakly positive cells for IL-11 were found in the mid-gestation spongiotrophoblast (Fig. 2g), whereas the columns of the labyrinth showed strong staining, clearly intracytoplasmic (Fig. 2h).

Later, on day 19, a contrast for anti-IL-11 staining was again noted between spongiotrophoblast (negative) and labyrinth (positive; Fig. 2j). However, it was less marked than the earlier one. The pattern was similar, with both areas opposed (Fig. 2i), but more homogeneous. Lastly, the outer giant cells were also stained (data not shown).

Interleukin-12

Uterine chamber (day 8·5). Anti-IL-12 staining was less strong than the anti-IL-11 one, and particularly heterogeneous (Fig. 3a). Some isolated stained areas were observed in the stroma, most of them around the implantation site. The staining was faint for the

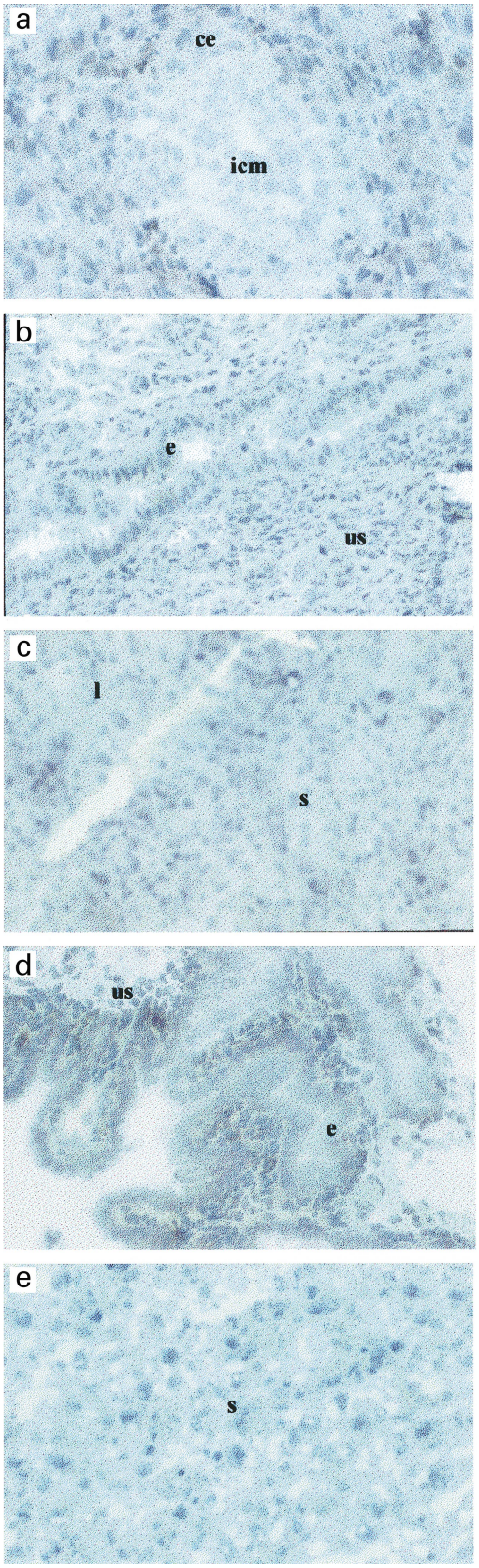


Table 1. Expression and distribution of IL-11, IL-13 (anti-inflammatory), IL-12 and IL-15 (pro-inflammatory) throughout murine pregnancy

	IL-11	IL-13	IL-12	IL-15
Early peri-implantatory phase (day 8·5)				
Inner cell mass	–	–	+/–	–
Ectoplacental cone	+	+ +/–	+/–	+
Primary giant cells	–	+	–	+
Metrial gland	+	inc.†	inc.	+/–
Stroma	+ +	+	+	+
Mid-gestation decidua (day 10·5)				
Glandular epithelium	+ + +	+ +	+ +	+
Stroma	+ +	+	+	–
Isolated cells*	+ +	n.f.‡	+ +	+/–
Mid-gestation placenta (day 10·5)				
Spongiotrophoblast	–	+ +	+ +	+ areas
Labyrinth	+ +	–	–	+/– areas
Foetal vessels	–	n.f.	+ +	+ or +/–
Reichert's membrane	+	+	–	inc.
Late decidua (day 19)				
Glandular epithelium	+ +	+/–	+/–	inc.
Stroma	+	–	–	inc.
Isolated cells*	+	n.f.	+	inc.
Late placenta (day 19)				
Spongiotrophoblast	+/–	–	+ spots	+ isolated cells
Labyrinth	+ +	–	+ spots	+ isolated cells

*Kind of cells to be determined.
†Inconclusive.
‡Not found on the studied slides for this pattern.

inner cell mass and the ectoplacental cone, and negative for giant cells (Fig. 3b).

Decidua. At mid-gestation, we observed the same pattern as for anti-IL-11, but it was attenuated (Fig. 3c): staining of the uterine epithelium (Fig. 3d) was less marked than in the uterine stroma.

Later (day 19), only uterine epithelial cells showed intracytoplasmic staining, and inner decidua seemed negative (Fig. 3e, f)

Placenta. It should be noted that we found at mid-gestation the opposite situation for anti-IL-12 labelling compared to anti-IL-11: a definite positive staining was observed in the spongiotrophoblast area (Fig. 3h) and the labelling of the labyrinth area was totally negative (Fig. 3g). Foetal vessels were also strongly stained (Fig. 3i).

At the later stage of pregnancy, the anti-IL-12 staining was less definite than earlier. Labyrinth and spongiotrophoblast zones both showed small areas of positive cells (Fig. 3k). The centre of the labyrinth was negative. The junction between labyrinth and spongiotrophoblast appeared positive (Fig. 3j).

Fig. 1. Negative controls throughout murine gestation. (a) Uterine chamber (day 8·5); decidualization phase, focus on the implantation site (icm: inner cell mass; ec: ectoplacental cone). (b) Mid-gestation decidua (day 10·5) (us: uterine stroma; e: glandular epithelium). (c) Mid-gestation placenta (day 10·5) showing both areas of placenta: spongiotrophoblast (s) and labyrinth (l). (d) Late decidua (day 19). (e) Late placenta (day 19) area. Original magnification, a–e × 200.

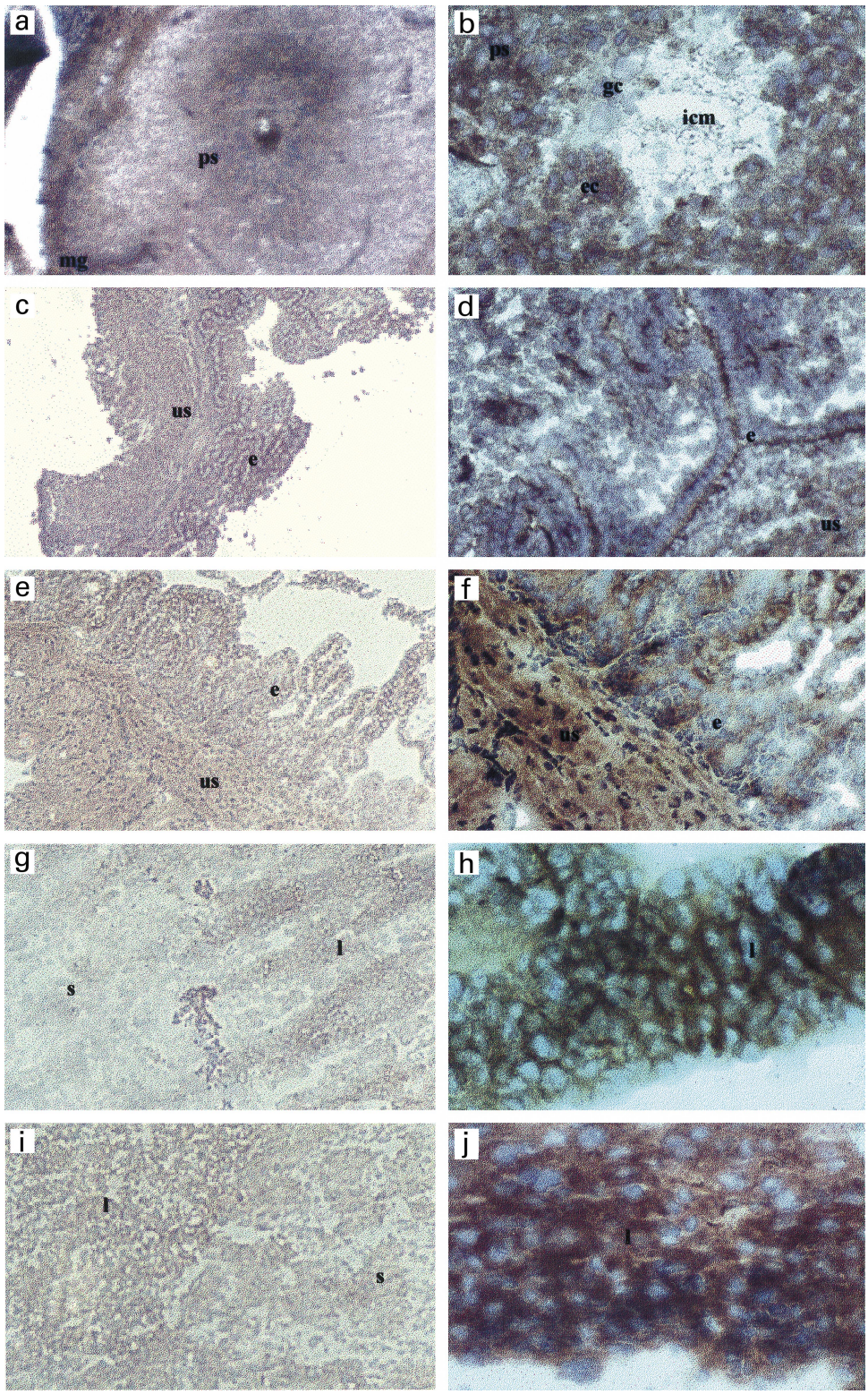
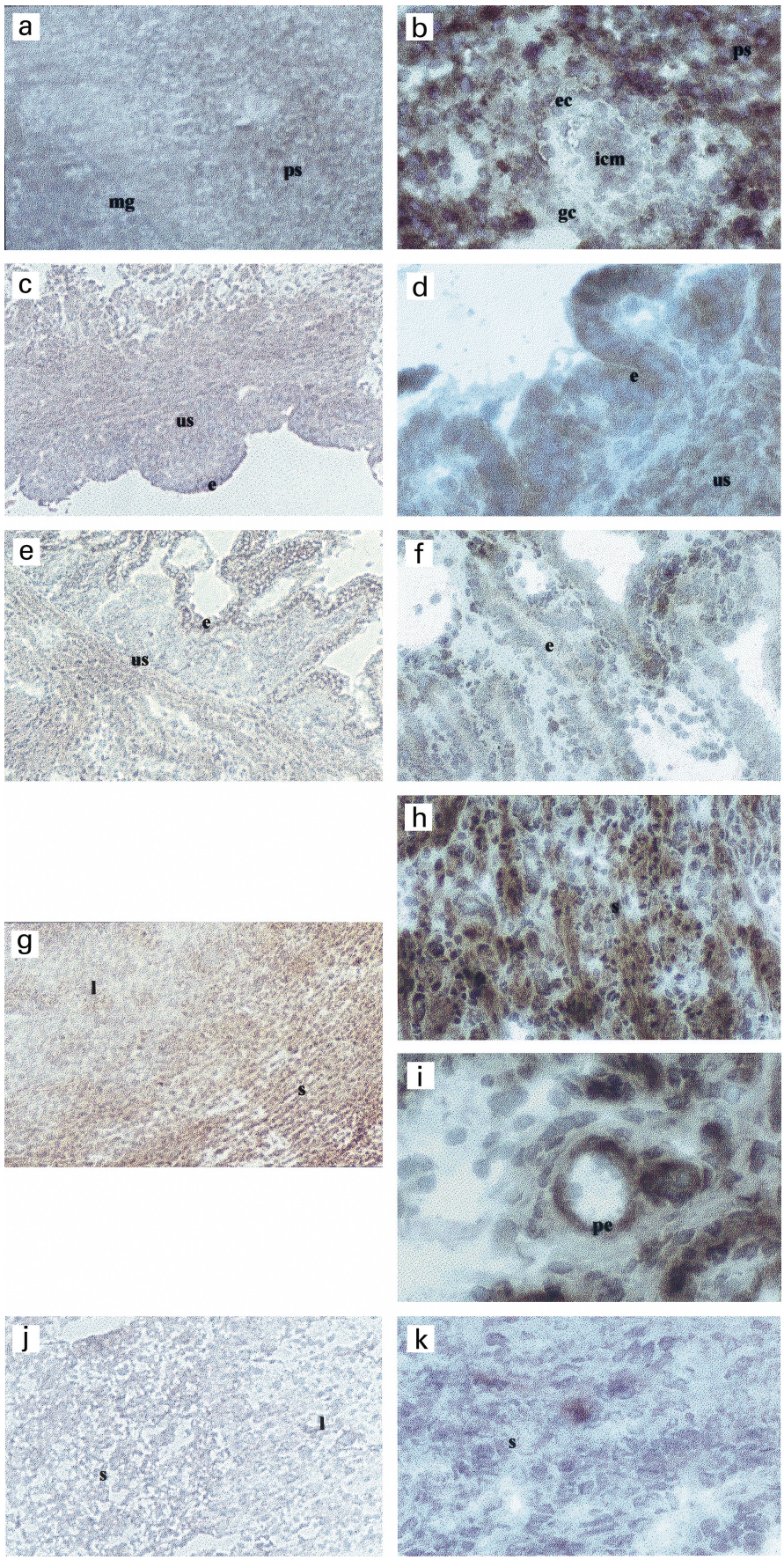


Fig. 2. Anti-IL-11 labelling of murine placentae and deciduae throughout gestation. (a) Decidualized uterine chamber (day 8·5) (ps: proliferative stroma; mg: metrial gland); (b) focus on the implantation site (gc: giant cells). (c) Mid-gestation decidua (day 10·5); (d) focus on the glandular epithelium. (e, f) Late gestation decidua (day 19). (g) Mid-gestation placenta (day 10·5); (h) focus on a column of the labyrinth. (i) Late gestation placenta (day 19); (j) focus on the labyrinth. Original magnification, a, c, e, g, i × 100; b, d, e, h × 200; j × 400.

Fig. 3. Anti-IL-12 labelling of murine placentae and deciduae throughout gestation. (a) Decidualized uterine chamber (day 8·5); (b) focus on the implantation site. (c) Mid-gestation decidua (day 10·5); (d) focus on the glandular epithelium. (e, f) Late gestation decidua (day 19). (g) Mid-gestation placenta (day 10·5); (h) focus on the spongiotrophoblast; (i) focus on stained placental vascular endothelium (pe). (j) Late gestation placenta (day 19); (k) isolated stained cells in the spongiotrophoblast. Original magnification, a, c, e, g, j × 100; b, f, h, k × 200; d, i × 400.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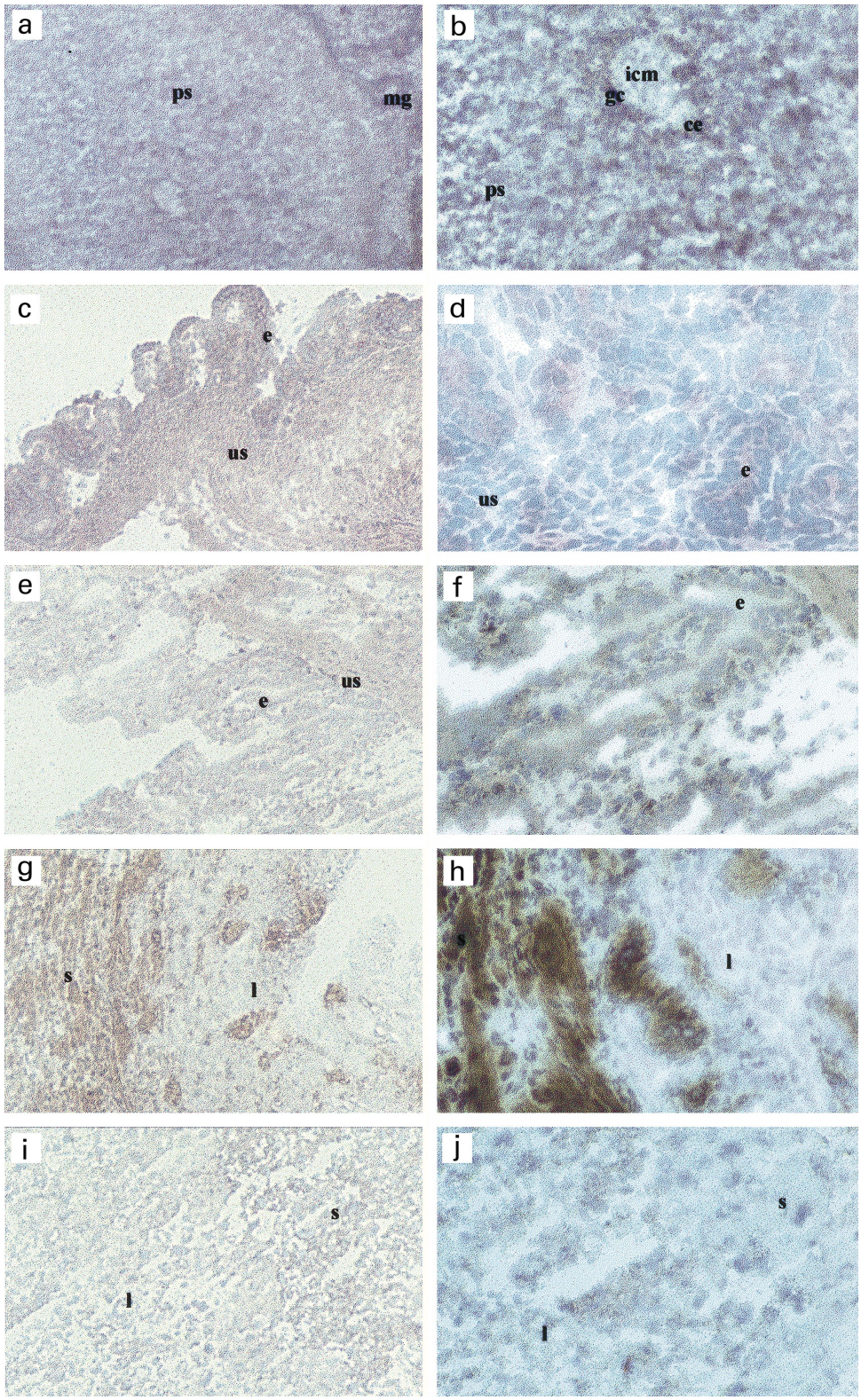

Fig. 4. Anti-IL-13 labelling of murine placentae and deciduae throughout gestation. (a) Decidualized uterine chamber (day 8·5); (b) focus on the implantation site. (c) Mid-gestation decidua (day 10·5); (d) focus on the glandular epithelium. (e, f) Late gestation decidua (day 19). (g, h) Mid-gestation placenta (day 10·5). (i, j) Late gestation placenta (day 19). Original magnification, a, c, e, g, i $\times$ 100; b, f, h, j $\times$ 200; d $\times$ 400.

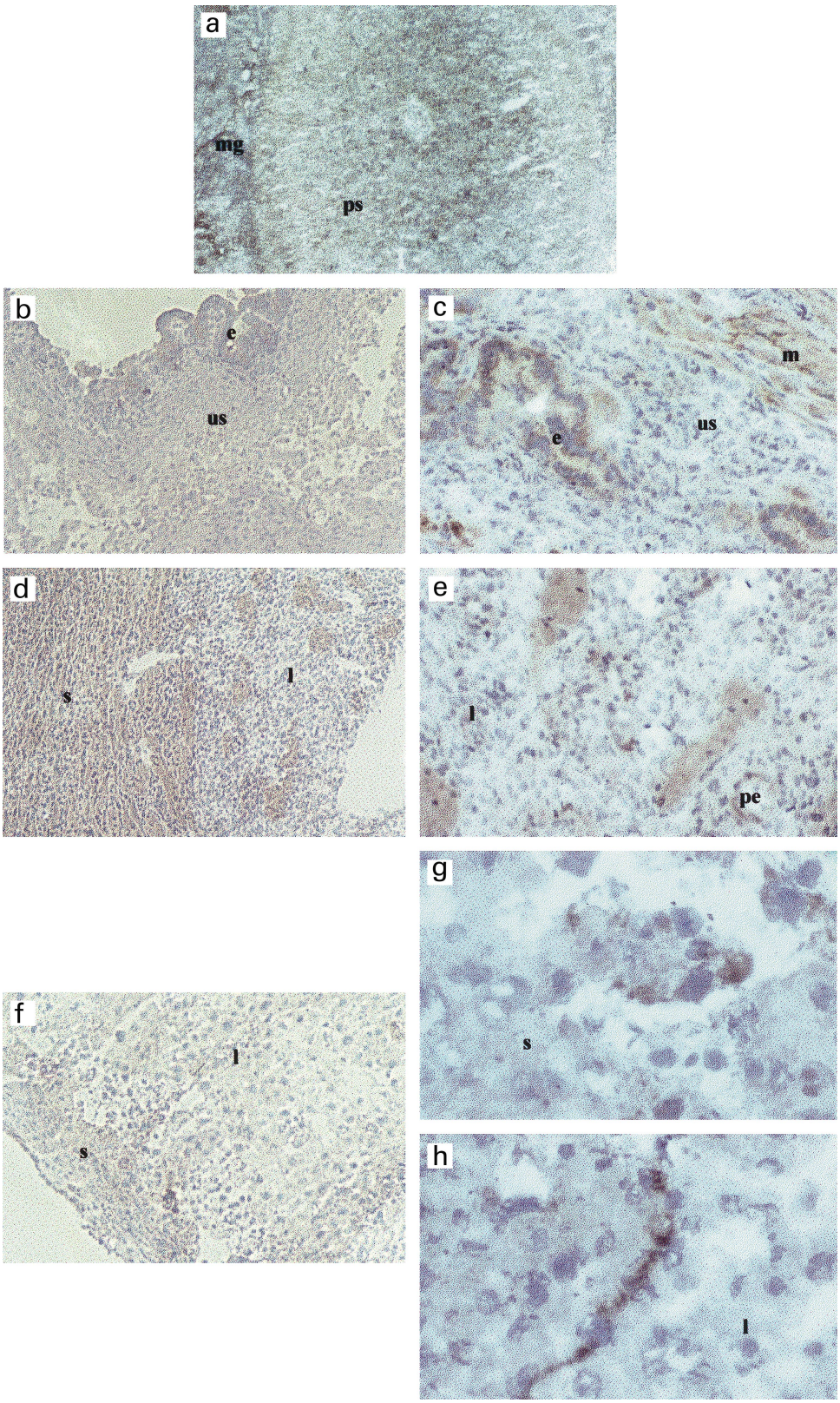

Fig. 5. Anti-IL-15 labelling of murine placentae and deciduae throughout gestation. (a) Decidualized uterine chamber (day 8·5). (b) Mid-gestation decidua (day 10·5); (c) focus on the glandular epithelium (m: uterine muscle). (d, e) Mid-gestation placenta (day 10·5). (f) Late gestation placenta (day 19); (g) isolated stained cells in the spongiotrophoblast; (h) infiltrated stained cells in the labyrinth. Original magnification, a, b, d, f × 100; c, e × 200; g, h × 400.

Interleukin-13

Uterine chamber (day 8·5). The labelling was scattered over different areas of the stroma (Fig. 4a). However, we found positive cells only around the implantation site. This positive area corresponded to the outline of the future embryo and its annexes (Fig. 4b). The inner cell mass was negative.

Decidua. In mid-gestation, we noted again a staining of the uterine epithelium (Fig. 4d), attenuated in the uterine stroma (Fig. 4c). This pattern was still diminished compared to anti-IL-11 and anti-IL-12.

Anti-IL-13 (Fig. 4e) staining was not obvious at day 19 in the decidua. Only a few isolated cells apparently showed intracytoplasmic staining (Fig. 4f).

Placenta. A similar pattern as for anti-IL-12 was observed (Fig. 4g), with a clear contrast between spongiotrophoblast (positive) and labyrinth (negative). However, a few positive cells from the spongiotrophoblast seemed to infiltrate the labyrinth area (Fig. 4h).

But later on, anti-IL-13 staining was nearly absent (Fig. 4j). The contact zone between spongiotrophoblast and labyrinth was possibly slightly positive (Fig. 4i).

Interleukin-15

Uterine chamber (day 8·5). Anti-IL-15 staining was faint and localized in two areas in the stroma: an outer weaker one, an inner one, which appeared more homogeneous and, as with anti-IL-13 labelling, outlined the embryo (Fig. 5a). No staining was observed in the embryo area, except a few positive cells next to the ectoplacental cone.

Decidua. The labelling was almost absent in the mid-gestation decidua. The glandular epithelium was lightly coloured by a diffuse intracytoplasmic staining (Fig. 5b). We were able to note a positive staining in uterine muscular areas (Fig. 5c).

On day 19, uterine stroma appeared negative for IL-15. A barely detectable positivity was found in the uterine glandular epithelium (data not shown).

Placenta. We noted on day 10·5 some positive areas within both spongiotrophoblast and labyrinth, with a diffuse intracytoplasmic staining (Fig. 5d, e). The pattern of staining was less definite than for the other interleukins.

Finally, the late placenta showed overall no definitely localized anti-IL-15 staining (Fig. 5f). Only a few isolated cells were labelled (Fig. 5g). We were even able to note a linear positive zone within the labyrinth (Fig. 5h), which we presumed were infiltrated lymphoid cells. Those cells were unequivocally labelled, being isolated (Fig. 6a) or in four to six cells clusters (Fig. 6b), by an anti-CD2 polyclonal antibody.

Within the limits of the sensitivity of this technique, and within the subjective approach it imposes, the signal seemed to enter a decline at the end of pregnancy. The peri-implantation stage was characterized by a more or less pronounced staining of the decidualized uterine stroma, whereas there was no evidence for a staining of the future conceptus. Lastly, the outstanding point in the mid-stage was the complementarity or the contrast of staining in the placenta with regard to the different interleukins, localized in different areas of the placenta.

DISCUSSION

Implantation and peri-implantation stages are correlated in mice and humans with a transient local inflammatory reaction [25], thought to be necessary to induce the expression of adhesion molecules and enzymes that control trophoblastic invasion and the attachment to uterine cells. However, after implantation, high levels of inflammatory cytokines appear to be abortifacient. Therefore, this inflammatory reaction is dampened, and the current paradigm emphasizes the role of the Th2 cytokines [2,3], whose distribution has been well documented in mice and humans, including by ourselves [26].

Much less is known about the expression of the more recently discovered cytokines, IL-11, IL-12, IL-13 and IL-15, especially in mice. We therefore conducted such a study by IHC in the murine placentas and deciduae of early, mid-pregnancy and late pregnancy obtained from syngenic (Balb/c × Balb/c) or allogenic matings (CBA/J × Balb/c). This study has been complemented by a second study dealing with IL-16, IL-17 and IL-18 (Ostojic *et al*. submitted).

IHC does not reveal obvious quantitative differences between the two mating combinations. This suggests that it would not be a good tool to compare abortion prone (CBA/J × DBA/2) and non-abortion prone (CBA/J × Balb/c) matings, as indeed has been confirmed by preliminary studies (Cayol, Mairovitz, Ostojic and Chaouat) [26] and unpublished sources.

These cytokines were therefore traced in the murine uterus and placenta, and show an anatomically and stage-dependent expression pattern which is specific for each cytokine, suggesting mutual or complementary controls.

Within the aforementioned limitations of IHC quantification, the peak expression of each of these interleukins seems to occur in the first half of murine pregnancy, followed by subsequent

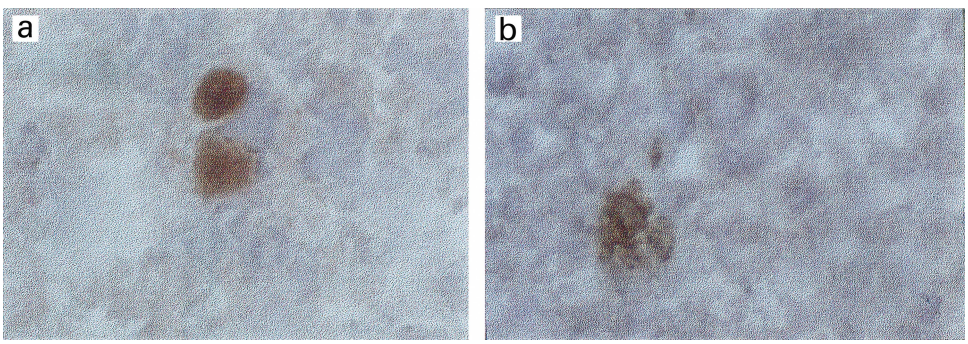

Fig. 6. Anti-CD2 labelling of the cytokine producing isolated lymphoid cells in late gestation placenta (double staining experiments). (a) Two isolated lymphoid cells. (b) Cluster of lymphoid cells (as most often observed). Original magnification a × 400; b × 200.

decline, especially for IL-12, IL-13 and IL-15. These results for IL-15 corroborate those obtained by RT–PCR [21].

Regarding maternal tissues, the uterine stroma was positive for all four interleukins in the peri-implantation stages.

The patterns were more specific regarding embryonic tissues. The ectoplacental cone exhibited strong IL-11 expression, corroborating *in situ* hybridization data [27,28]. IL-12, IL-13 and IL-15 were found in the ectoplacental cone, but only IL-13 and IL-15 appeared in the primary giant cells.

The expression of these four cytokines in murine placentae in mid-gestation is also striking. While IL-12 and IL-13 were located within the spongiotrophoblast zone, in a complementary pattern, the expression of IL-11 was restricted to the labyrinth zone. Such a complementary or 'mirror-image' expression of IL-12, IL-11 and IL-13 which display, respectively, pro- and anti-inflammatory properties, suggests, but does not prove, a mutual regulation. Indeed, the space–time expression of IL-13 corresponds to the one we have observed for IL-10 [26], in keeping with its anti-inflammatory activities. Thus, IL-13 might complement or replace IL-10 as a backup mechanism, and could explain generally normal pregnancy in IL-10 knock out mice.

The expression of IL-11, IL-12, IL-13 and IL-15 at the foetomaternal interface, while it suggests or confirms a putative role for them during murine gestation, does not seem fully compatible with the paradigm of pregnancy viewed as a pure Th1/Th2 concept [2,3,26]. As useful as it has been, this model appears now to be an oversimplification, even if one incorporates the Th0 concept. Other recently published data raise questions about this Th1/Th2 paradigm [29,30], since interferon-tau [31], Colony Stimulating Factors [32], LIF [33], which are essential for pregnancy, do not fit fully or even partially into such a dichotomy.

Here, even if one can generally distinguish pro-inflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines, other roles than involvement in the Th1/Th2 are to be noted.

For example, decidualization defects in IL-11 receptor alpha (IL-11Rα) knock-out mice [27,28], resulting in the absence of successful gestation beyond day 12, are attributed to abnormal decidual cell transformation, leading to a disruption of the expression of inhibitors of proteases [34,35], and trophoblast invasion, causing an anarchical proliferation of the latter, and thus pregnancy failure. IL-11 could also have a paracrine or autocrine function because of the space–time expression of ligand and receptor in the foetal and maternal tissues [27,28]. Lastly, as a growth factor for epithelial cells, and through its action on vascular endothelium, IL-11 could also be involved in the proliferation of the decidual stroma and the remodelling of spiral arteries. An effect on the ectoplacental cone itself should not be excluded, even though a contradiction between elevated ectoplacental cone volumes and reduced mitotic indexes appears in studies conducted in IL-11 treated rats [36].

IL-12 causes abortion when injected with IL-18 [20], most likely by activating NK cells or by controlling T cell cytotoxicity [37,38]. At physiological doses, it could control trophoblast invasion in the decidua. Moreover, IL-12 acts on angiogenesis [39], and it could be involved in the vascular remodelling that occurs during placentation: mice with a mutation towards IL-12 or components of its signal transduction [40] showed a vascular pathology at the uteroplacental interface. This latter property could also be related to IL-12 effects on IFN-γ production since IFN-γ appears to contribute to normal health of the mid-gestational decidua, possibly via NK production of angiogenic molecules such as angiopoietin II [40].

Zonal expression of IL-13, as well as the extinction of the signal after mid-gestation that we observed in mice, are in complete concordance with that observed during human pregnancy [41]. IL-13 is there thought to be required during the first third of gestation to support trophoblast invasion within the decidua.

Finally, regarding IL-15, studies of its receptor demonstrate its presence in the pregnant uterus, whereas the IL-2 receptor is not present [21]. IL-2 and IL-15 functions are often related [42] because their receptors share one chain, but the expression pattern we observed suggests a distinct immunoregulatory role for IL-15 during pregnancy. IL-15, known to stimulate NK cells [24], could regulate the differentiation of the uterine NK cells [43] and their secretion of cytolytic mediators [44].Thus, an abnormally high expression of IL-15 could lead to abortion. In this context, in human preterm labour, a higher IL-15 concentration is observed in the amniotic fluid and decidua [45].

IL-15 could also have a distinct effect on trophoblast, regulating its invasion by increasing collagenolytic activity and metalloproteinases expression [24], known to be involved in this process [46]. Lastly, IL-15 is supposed to inhibit apoptosis [47], which is essential to the remodelling related to placental development [48].

These functions are still poorly understood, even if our data suggest such a role for IL-11, IL-12, IL-13 and IL-15, because data are more scarce in this respect in mice than in humans. As the expression of these molecules has now been demonstrated, further studies are required to confirm the hypothesis we have suggested.

First, it will be necessary to refine our immunohistochemical data by double-labelling IHC. As far as the function itself is concerned, we believe that IHC cannot *per se* address this question directly, because of the difficulty in comparing in an unbiased fashion the cytokine expression levels. The comparative studies between normal gestating (CBA × BALB/c) and abortion-prone (CBA/J × DBA/2) [49] murine mating combinations, need to be performed by a combination of ELISA and quantitative RT–PCR, as well as *in vivo* immune neutralization on the one hand and high-dose injection on the other hand. As an example, we already find more IL-12 in the abortion prone CBA × DBA/2 mating combination than in the nonabortive one at the onset of the resorption window (data not shown), but the full completion of such studies, now in progress, are far beyond the brief of this first publication, which deliberately limited itself to a complete longitudinal study of cytokine presence and expression.

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