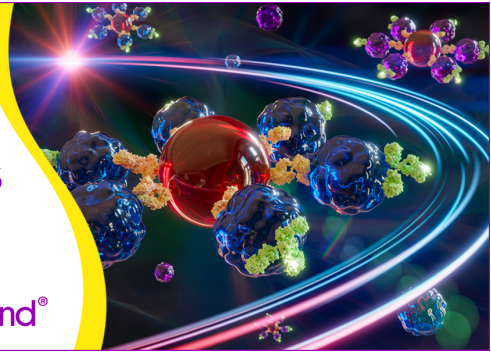


Advance allergy and  
asthma research by  
analyzing multiple cytokines  
with LEGENDplex™

Start multiplexing ▶

BioLegend®



## The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 15 2005

### Intercellular Adhesion Molecule-1/LFA-1 Cross Talk Is a Proximate Mediator Capable of Disrupting Immune Integration and Tolerance Mechanism at the Feto-Maternal Interface in Murine Pregnancies<sup>1</sup> **FREE**

Sandra Blois; ... et. al

*J Immunol* (2005) 174 (4): 1820–1829.

<https://doi.org/10.4049/jimmunol.174.4.1820>

#### Related Content

Erythroid suppressor cells promote feto-maternal tolerance through arginase-2 and PDL-1

*J Immunol* (May,2018)

*Extracellular vesicles deliver feto-placental paternal antigens to maternal secondary lymphoid tissues for T cell recognition.*

*J Immunol* (May,2023)

A Microphysiological Device to Model the Chorionic Interface Immune Status during Pregnancy

*J Immunol* (March,2023)

# Intercellular Adhesion Molecule-1/LFA-1 Cross Talk Is a Proximate Mediator Capable of Disrupting Immune Integration and Tolerance Mechanism at the Feto-Maternal Interface in Murine Pregnancies<sup>1</sup>

Sandra Blois,\*<sup>†</sup> Mareike Tometten,\* Judith Kandil,\* Evelin Hagen,\* Burghard F. Klapp,\* Ricardo A. Margni,<sup>†</sup> and Petra C. Arck<sup>2\*</sup>

Our understanding why a woman's immune system does not reject her histoincompatible fetus is still very limited. Distinct insights into the mechanisms involved in pregnancy maintenance may help us to prevent pregnancy complications, e.g., miscarriages or pre-eclampsia. Immune integration and tolerance at the feto-maternal interface appear to be indispensable for successful pregnancy maintenance. Little is known about the cross talk between ICAM-1, expressed on epithelium, endothelium, and APC, and its ligand, LFA-1, at the feto-maternal interface. However, based on the role of ICAM-1/LFA-1 in allograft acceptance or rejection upon transplantation, adhesion molecules are likely to interfere with successful pregnancy outcome. In this study, we tested the hypothesis that ICAM-1/LFA-1 pathways may be involved in pregnancy rejection in murine models. By blocking ICAM-1/LFA-1-mediated intercellular adhesion events, we show that fetal immune acceptance is restored in challenged pregnancies (e.g., upon exposure to sound stress), and adoptive transfer of LFA-1 cells into pregnant mice induces rejection only in abortion-prone mouse models. ICAM-1/LFA-1 cross talk leads to increased recruitment of proinflammatory cells to the implantation site, promotes dendritic cell maturation in the decidua, and subsequently induces additional local Th1 polarization via mature dendritic cells. Furthermore, our observations clearly point out that mechanisms of fetal tolerance, e.g., indoleamine 2,3-dioxygenase expression, presence of CD4<sup>+</sup>CD25<sup>bright</sup> regulatory T cells, and synthesis of asymmetric Abs, are ICAM-1/LFA-1 dependent. Hence, our data shed light on a hierarchical network of immune integration at the feto-maternal interface, in which ICAM-1/LFA-1 cross talk is clearly a proximate mediator capable of disrupting successful pregnancy maintenance. *The Journal of Immunology*, 2005, 174: 1820–1829.

Successful blastocyst implantation and early placental development are the result of complex regulatory mechanisms. At a functional level, the developing placenta must integrate maternal and fetal physiology, whereby spatial adjacencies at the interface of fetal and maternal tissues guarantee nourishment of the fetus (1). Intriguingly, such contiguousness of fetal tissue carrying paternal Ags, and maternal tissue does generally not provoke fetal rejection via maternal immune cells (1, 2). Hence, it has been suggested that plural tolerance mechanisms have evolved to ensure the maintenance of the feto-placental graft. Such mechanisms include the following: 1) the predominance of anti-inflammatory, Th2 cytokines over proinflammatory Th1 cytokines in the decidua (3–5); 2) the decidual expression of in-

doleamine 2,3-dioxygenase (IDO),<sup>3</sup> an enzyme that furnishes immune rejection by depriving the T cells of tryptophan and/or by inhibiting lymphocyte proliferation (6–8); 3) the presence of CD4<sup>+</sup>CD25<sup>bright</sup> regulatory T (Treg) cells, which suppress an aggressive allogeneic response directed against the fetus, as simultaneously described in human pregnancies by the Saito group (9) and in murine pregnancies (10); and 4) the synthesis of asymmetric IgG Abs (AABs), which have been suggested to camouflage paternal Ags expressed by the placenta (11–13). In addition, it has become apparent in the past few years that dendritic cells (DCs) seem to be an essential regulatory cell subset at the feto-maternal interface in mediating tolerance (14–16).

Distinct subsets of DCs, besides macrophages and B cells, may serve as professional APC (17). To facilitate T cell interaction with APC, leukocytes that circulate as nonadherent cells can be recruited to a specific site of inflammation, e.g., to the blastocyst invasion site. The process of recruitment first involves T cells rolling along the surface of vascular endothelial cells, followed by the arrest and, finally, migration of T cells into tissue (18). Molecules that play a proximate and critical role in controlling tethering (adhesion) and spreading of T cells are integrins such as LFA-1 (CD11a/CD18,  $\alpha_1\beta_1$  integrin) (18, 19). Blocking of LFA-1 by injection of mice with mAb against LFA-1 in vivo has been described to prevent fetal rejection (20), to reduce the severity of graft-vs-host reactions (21), to prolong allograft survival (22), to

\*Charité, University Medicine Berlin, Berlin, Germany; and <sup>†</sup>Humoral Immunity Studies Institute, National Council of Scientific and Technological Research, University of Buenos Aires, Buenos Aires, Argentina

Received for publication August 13, 2004. Accepted for publication November 11, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This study was supported by grants from the Deutsche Forschungsgemeinschaft (AR 232/8-1), the Fritz-Bender Foundation, and Charité (to P.C.A.); S.B. received a scholarship from the German Student Exchange Program (Deutscher Akademischer Austauschdienst).

<sup>2</sup> Address correspondence and reprint requests to Dr. Petra C. Arck at her current address: Biomedizinisches Forschungszentrum, Raum 2.0549, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail address: petra.arck@charite.de

<sup>3</sup> Abbreviations used in this paper: IDO, indoleamine 2,3-dioxygenase; Treg, regulatory T; AAB, asymmetric Ab; DC, dendritic cell; Gd, gestation day; RT, room temperature; MHC-II, MHC class II; HPRT, hypoxanthine phosphoribosyltransferase.

inhibit the development of autoimmunity (23), and to block neuropeptide substance P-induced leukocyte migration (24). The latter observation is particularly striking because our previous findings revealed that the lack of immune tolerance, similar to graft-vs-host reactions, is mediated via substance P in stress-triggered murine pregnancy failure (25).

ICAM-1 (CD54), a 95-kDa member of the Ig superfamily found on lymphocytes, vascular endothelium, high endothelial venules, epithelial cells, macrophages, and DC is a ligand for LFA-1 (18, 26). Its expression is up-regulated upon stimulation by inflammatory mediators such as cytokines and LPS and subsequently facilitates a selective recruitment of leukocytes in a variety of pathological states (17, 18). ICAM-1-mediated intercellular adhesion events, e.g., the firm adhesion of T cells to epithelial, endothelial, or APC cells, can be blocked by injection of mAb against ICAM-1 *in vitro* and *in vivo* (17, 26, 27).

Lack of immune tolerance and subsequent rejection of the fetus are a common and fatal pregnancy complication, e.g., miscarriages affect 15–40% of pregnancies in humans (1, 2). Furthermore, the verdict of survival or rejection of the fetus is partially dependent on the events endangering the mother, prevalently environmental parameters such as exposure to psychoemotional stress (5). Based on their importance in both cell recruitment and cross talk with APC, LFA-1 and ICAM-1 molecules may become attractive targets for developing new therapies to facilitate fetal tolerance by preventing rejection pathways during pregnancy, e.g., triggered by stress exposure. A growing understanding of the function of LFA-1 and ICAM-1 in reproduction and the mechanism of their interaction with tolerance mechanisms could make it possible to develop therapies based on blocking these adhesion molecules by application of Abs.

Consequently, in the present study, we investigated the expression of adhesion molecules and their functional role in the pregnancy outcome by using an abortion-prone mating combination, DBA/2J-mated CBA/J females, and low abortion matings, BALB/c-mated CBA/J females. Interestingly, when exposed to sound stress during early gestation, DBA/2J-mated CBA/J females, but not BALB/c-mated CBA/J females, show an increased abortion rate, which allows investigation of pregnancy pathophysiology similar to human miscarriages (25).

We addressed the following key questions: 1) Does the expression of LFA-1 and ICAM-1 change in uterine tissue from stress-triggered, pathologic pregnancies compared with unchallenged pregnancies? 2) Does blocking of ICAM-1/LFA-1-mediated intercellular adhesion events abolish the detrimental effects of stress on abortion rate, Th1/Th2 ratio, and tolerance mechanisms (IDO expression, Treg cells, and AAbs)? 3) Do adhesion molecules induce an increased migration of different leukocyte subsets into decidual tissue in pathological pregnancies? 4) Do adhesion molecules affect phenotype of DCs present at the fetomaternal interface? 5) Does the transfer of LFA-1<sup>+</sup> cells to CBA/J females in abortion-prone mating combination (mated to DBA/2J males) or low abortion mating combination (mated to BALB/c males) mimic the stress effects?

## Materials and Methods

### Animals

Mice were purchased from Charles River and maintained in an animal facility with a 12-h light/dark cycle. Animal care and experimental procedures were followed according to institutional guidelines and conformed to requirements of the state authority for animal research conduct (LaGetSi, Berlin, Germany).

We performed three experiments. Experiment I was to investigate the effect of stress on adhesion molecule expression (ICAM-1, LFA-1) in DBA/2J-mated CBA/J females. The mice were divided into control and

stress group (exposure to stress, see below). The pregnant females were sacrificed on gestation days (Gd) 7.5 and 10.5. Uteri were removed and divided into pieces. One section was frozen for immunocytochemistry or immunofluorescence, and from another section, uterus cells were isolated for flow cytometry.

Next, experiment II was performed to analyze the effect of blocking of ICAM-1/LFA-1-mediated intercellular adhesion events involved in immune responses in DBA/2J-mated, stressed CBA/J females. Females with vaginal plugs were again segregated and divided into different subgroups: one group served as a control, receiving 100  $\mu$ l of sterile PBS by *i.p.* injection ( $n = 5$ ) from Gd 2.5 to 6.5. Another group was exposed to stress on Gd 5.5 and received *i.p.* injections of 100  $\mu$ l of sterile PBS from Gd 2.5 to 6.5. The third group was exposed to stress on Gd 5.5 and injected with mAbs against ICAM-1 and LFA-1 from Gd 2.5 to 6.5. We used a mixture of anti-LFA-1, which reacts with the 180-kDa  $\alpha_1$ -chain of LFA-1, a heterodimeric surface glycoprotein expressed on almost all leukocytes (clone M17/4; catalog no. 553337; BD Pharmingen), and anti-ICAM-1 (clone 3E2; catalog no. 553249; BD Pharmingen), and injected 25  $\mu$ g of each mAb/mouse/day in 100  $\mu$ l of sterile PBS.

One-half of the mice per group were sacrificed on Gd 7.5 to harvest uterine cells for subsequent characterization of surface marker and analysis of intracellular cytokines by flow cytometry. The remaining mice per group were sacrificed on Gd 13.5, because the abortion rate (percent resorption to total number of implants) may be macroscopically evaluated on this Gd.

Then, experiment III was performed to investigate the effect of LFA-1<sup>+</sup> blood cells from stressed DBA/2J-mated CBA/J females transferred into two different models of murine pregnancy: 1) BALB/c-mated CBA/J females (low abortion mating combination) and 2) DBA/2J-mated CBA/J females (abortion-prone mating combination). The respective females received MACS-enriched LFA-1<sup>+</sup> blood cells *i.v.* on Gd 6.5. The mice were sacrificed on Gd 7.5 and 13.5, and the same experimental procedures were performed as in experiment II.

### Application of stress

The females were exposed to sound stress for the duration of 24 h starting on Gd 5.5. The sound stress was emitted by a rodent repellent device (Conrad Electronics) at a frequency of 300 Hz in intervals of 15 s. The stress device was placed into the mouse cage so that the mice could not escape the sound perception.

### Immunohistochemical staining for ICAM-1

Cryostat sections (8  $\mu$ m) were incubated with peroxidase-, avidin-, and biotin-blocking solution (Vector), followed by another block using protein blocking agent (Immunotech). The biotinylated hamster anti-mouse ICAM-1 mAb (no. 01542D; BD Pharmingen) was diluted 1/100 in TBS containing 1% FCS and applied for 1 h. As amplification and revealing system, we used peroxidase complex (Vector) 1:100 in TBS for 30 min. The signal was detected by incubating sections with 0.2 mg/ml diaminobenzidine (Sigma-Aldrich) and 0.05% hydrogen peroxide, followed by light counterstaining with 0.1% Mayer's hematoxylin. The signal intensity was scored as follows: -, negative; +/-, patchy; +, weak; ++, moderate; +++, high. Slides were examined using a Zeiss Axioscope light microscope. Photodocumentation was performed using digital image analysis system (Zeiss KS400).

### Preparation of uterus cell suspensions

To obtain suspensions of uterine cells for FACS, a method described previously (16, 28) was used. Briefly, uteri were collected, washed with sterile PBS, carefully cut into small pieces, collected in tubes containing HBSS, and digested for 20 min at 37°C under slight agitation in HBSS with 200 U/ml hyaluronidase (no. H3506; Sigma-Aldrich), 1 mg/ml collagenase (type C-2139; Sigma-Aldrich), 0.2 mg/ml DNase I (no. 1284932; Boehringer Mannheim), and 1 mg/ml BSA/fraction V (no. A9418; Sigma-Aldrich). Thereafter, the isolated cells were collected in a fresh tube through a 100- $\mu$ m net (BD Biosciences) and washed with RPMI 1640/10% FBS. The procedure was repeated twice, with HBSS medium containing no mixture of enzymes. Cells were resuspended in a 1.080 g/cm<sup>3</sup> HistoDenz solution (no. H5528; Sigma-Aldrich) and centrifuged at 800  $\times$  g for 20 min at room temperature (RT). The low density fraction at the interface was collected and washed several times.

### Purification of dendritic uterine cells

To identify DC subsets, we isolated the CD11c<sup>+</sup> cell fraction by using magnetic cell sorting. For collection of cells expressing CD11c to investigate in experiments II and III, uterine suspensions were incubated (30 min, 4°C) with biotinylated hamster anti-mouse CD11c diluted 1/100 in

labeling buffer (PBS supplemented with 2 mM EDTA (no. L2113). After washing, cells were incubated (15 min, 4°C) with streptavidin MicroBeads (no. 130-048-101) and processed using MACS to collect CD11c<sup>+</sup> cells. Examination by FACS revealed that >96% of selected cells by mini-MACS expressed CD11c.

#### Flow cytometry analysis

Flow cytometry was performed using our standard protocol (16, 28). All mAbs were purchased from BD Biosciences and are listed in Table I. Briefly, uterus cells were washed twice with buffer (PBS supplemented with 1% BSA (no. A9418; Sigma-Aldrich) and 0.1% sodium acid (no. K32996788; Merck). Two percent of normal mouse serum was added to avoid nonspecific binding by FcRs. Cells were then incubated for 30 min at 4°C with the respective mAb against surface Ags (experiment I, LFA-1; experiments II and III, CD8 $\alpha$ , MHC class II (MHC-II), CD80, CD4, CD25, F4/80, TCR- $\gamma\delta$ , and NK1.1). After the cells were washed and fixed using Fix solution (no. 340181; BD Biosciences), they were incubated for 30 min at 4°C in the dark. Subsequently, the cells were washed and permeabilized, using FACS Permeabilizing Solution (no. 340973; BD Biosciences), followed by incubation with the respective Abs against intracellular Ags (experiments II and III, TNF- $\alpha$ , IL-4, IFN- $\gamma$ , IL-12, and IL-10) for 30 min at 4°C in the dark. Afterward, the cells were washed and then read. As controls, cells were stained with the corresponding isotype-matched mAb. The acquisition was performed using a FACSCalibur (BD Biosciences). Instrument compensation was set in each experiment using single-color stained samples. Data were analyzed by using CellQuest software. FACS results were expressed as percentage of cells positive for the surface marker evaluated.

#### Purification and phenotype of LFA-1 from blood cells

To purify LFA-1<sup>+</sup> cells for subsequent adoptive transfer, blood samples from DBA/2J-mated, stressed CBA/J females were obtained aseptically by retro-orbital puncture. Blood was collected in heparinized tubes. After treatment with sterile ammonium chloride lysis buffer for 10 min to deplete erythrocytes, the cells were washed twice with sterile PBS. The remaining cells were incubated (30 min, 4°C) with biotinylated rat anti-mouse LFA-1 (no. 557365) diluted 1/100 in labeling buffer. After washing, cells were incubated (15 min, 4°C) with streptavidin MicroBeads and processed using mini-MACS to collect LFA-1<sup>+</sup> cells. Examination by flow cytometry revealed that >95% of selected cells expressed LFA-1. The characteristics of LFA-1 blood cells used for the adoptive transfer in DBA/2J- and BALB/c-mated nonstressed CBA/J females were as follows: 66.8  $\pm$  2.0% CD3<sup>+</sup>, 21.7  $\pm$  1.8% CD8<sup>+</sup>, 1.0  $\pm$  0.1% NK1.1<sup>+</sup>, 0.25  $\pm$  0.1%  $\gamma\delta$  TCR<sup>+</sup>, and 0.6  $\pm$  0.1% CD11c<sup>+</sup>. The Th1/Th2 ratio was 8; it has been calculated by the sum of LFA-1<sup>+</sup>/TNF- $\alpha$ , LFA-1<sup>+</sup>/IFN- $\gamma$ , and LFA-1<sup>+</sup>/IL-12 cells, which was then divided by 3 to obtain the value for "Th1." The value for "Th2" is the sum of LFA-1<sup>+</sup>/IL-4 and LFA-1<sup>+</sup>/IL-10, divided by 2. Then, the Th1 value has been divided by the Th2 value.

#### LFA-1<sup>+</sup> cell phenotype and adoptive transfer

The characteristics of LFA-1 blood cells used for the adoptive transfer in DBA/2J- and BALB/c-mated, nonstressed CBA/J females were as follows: 66.8  $\pm$  2.0% CD3<sup>+</sup>, 21.7  $\pm$  1.8% CD8<sup>+</sup>, 1.0  $\pm$  0.1% NK1.1<sup>+</sup>, 0.25  $\pm$  0.1%  $\gamma\delta$  TCR<sup>+</sup>, and 0.6  $\pm$  0.1% CD11c<sup>+</sup>. The Th1/Th2 ratio was 8; it has been calculated by the sum of LFA-1<sup>+</sup>/TNF- $\alpha$ , LFA-1<sup>+</sup>/IFN- $\gamma$ , and LFA-1<sup>+</sup>/IL-12 cells, which was then divided by 3 to obtain the value for "Th1." The value for "Th2" is the sum of LFA-1<sup>+</sup>/IL-4 and LFA-1<sup>+</sup>/IL-10, divided by 2. Then, the Th1 value was divided by the Th2 value.

LFA-1 blood cells were incubated with 5  $\mu$ M CFSE solution (10<sup>7</sup> cells/ml; no. C1157; Molecular Probes) for 30 min at RT. The reaction was stopped by washing the cells three times with sterile PBS. Before LFA-1<sup>+</sup> cell transfer, cell viability was checked and adjusted to a concentration of 5  $\times$  10<sup>6</sup> cells/100  $\mu$ l with sterile PBS and i.v. injected into the recipients of experiment III on Gd 6.5.

#### Serum

On Gd 7.5, mice of experiments II and III were narcotized, and blood samples were taken by retro-orbital puncture. Serum samples were obtained by centrifugation and kept at -80°C until use.

#### Determination of asymmetric IgG molecules (AAbs)

Separation of symmetric and asymmetric IgG molecules was performed by Con A Sepharose chromatography, based on the fact that the lectin Con A binds asymmetric IgG molecules containing  $\alpha$ -D-mannopyranosyl,  $\alpha$ -D-glucopyranosyl, and sterically related residues. In brief, Con A-Sepharose (no. C9017; Sigma-Aldrich) was washed with elution buffer (25 mM Tris-HCl; 0.2 M NaCl; 3 mM each of CaCl<sub>2</sub>, MgCl<sub>2</sub>, MnCl<sub>2</sub>; and 0.02% Na<sub>3</sub>N (pH 7.2)). Equal volumes (100  $\mu$ l) of either 50% packed Con A-Sepharose or buffer were added to a 1/100 dilution of each serum sample, mixed in tubes, and incubated for 2 h at 4°C with occasional shaking. After centrifugation, both samples (containing the Con A-adsorbed and the nonadsorbed fractions, respectively) were tested in an ELISA to determine the IgG Abs. For analyzing IgG, a capture assay was used. Briefly, microtiter plates (Nunc Maxisorp; Immunoplates) were coated with 1  $\mu$ g/ml rabbit anti-mouse IgG (no. CH695795; Pierce), and blocked with 3% BSA (no. A7030) in PBS for 2 h at RT. Serial dilutions of each sample (containing the Con A-adsorbed and the nonadsorbed fractions, respectively) were added in duplicate and incubated for 2 h. As secondary Ab, a peroxidase-labeled rabbit anti-mouse IgG (no. P0260; Vector Laboratories) was used. The assay was developed by addition of *o*-phenylenediamine (no. P1526; Sigma-Aldrich), 1 mg/ml in citrate-phosphate buffer (pH 5) plus 0.3% H<sub>2</sub>O<sub>2</sub>. The percentage of asymmetrically glycosylated IgG was calculated as follows: % IgG bound to Con A (AAbs) = 100 - (nonbound IgG/total IgG  $\times$  100).

#### Total RNA isolation

For molecular biology studies, ~50 mg of uterine tissue was washed carefully with sterile PBS (pH 7.40), snap frozen in liquid nitrogen, and stored at -80°C until RNA isolation. After that, the tissue has been treated with 500  $\mu$ l of TRIzol (Invitrogen Life Technologies) and desegregated using a homogenizator (Pellet pestle; Sigma-Aldrich). The RNA was then extracted with chloroform, precipitated with absolute ethanol, washed, and finally diluted in sterile aqua dest. The integrity of the extracted RNA was evaluated by electrophoresis in ethidium bromide-stained 1.5% agarose gels. The purity of the extracted RNA was checked using the ratio between the OD at 260 and 280 nm, whereas the quantification was done taking into account that 1 OD at 260 nm equals 40  $\mu$ g of RNA.

#### Reverse transcription

After RNA preparation, the total RNA was treated with DNase I (1 U/1  $\mu$ g of RNA; Invitrogen Life Technologies) for 15 min at RT followed by inactivation with EDTA (Sigma-Aldrich). First-strain cDNA synthesis was performed using SuperScript II reverse transcriptase (Invitrogen Life Technologies) as follows: 2  $\mu$ g of the extracted RNA was incubated with 2  $\mu$ l of Random Hexamer Primers (Invitrogen Life Technologies) for 10 min at 70°C, denatured at 4°C for 1 min, and then 5  $\mu$ l of dNTPs (Fermentas), 4

Table I. List of mAbs used for flow cytometry<sup>a</sup>

Surface Ab against	Label	Catalog No.	Intracellular Ab against	Label	Catalog No.
LFA-1	PE	553121	IL-12	PE	554479
F4/80	FITC	RM2901	IL-10	PE	554467
TCR- $\gamma\delta$	FITC	553177	IL-4	PE	554435
CD4	FITC	553047	TNF- $\alpha$	PE	554419
NK1.1	Biot.	553163	IFN- $\gamma$	PE	554412
CD80	PE	553769	IL-12	PE	554479
CD25	PE	01105A			
MHC-II	PE	553544			
CD11c	Biot.	553800			

<sup>a</sup> All purchased at BD Pharmingen. Biot., Biotinylated.



$\mu$ l of first-strand buffer (Invitrogen Life Technologies), 2  $\mu$ l of DTT (Invitrogen Life Technologies), 1  $\mu$ l of the reverse transcriptase enzyme (SuperScript II; PerkinElmer), and water were added. The 20- $\mu$ l tubes were incubated for 10 min at RT, 1 h at 42°C, and 10 min at 70°C. The cDNA was used for real-time PCR. Control tubes included omission of SuperScript, of the first-strand buffer.

#### Real-time PCR for IDO

Real-time PCR was used to obtain quantitative data on differences between IDO mRNA of the control, stressed, anti-adhesion molecule-treated mice and also CBA/J  $\times$  DBA/2J or CBA/J  $\times$  BALB/c transferred LFA-1<sup>+</sup> mice. This assay exploits the 5'-nuclease activity of AmpliTaq Platinum (Invitrogen Life Technologies) DNA polymerase to cleave a fluorescent probe designed for IDO (TipMolBio), and to normalize our samples, a fluorescent probe for the housekeeping gene hypoxanthine phosphoribosyltransferase (*Hprt*) was used in real-time PCR. The sequences were as follows: *Hprt* primers, 5'-CAC AGG ACT AGA ACA CCT GC-3' and 5'-GTT GGA TAC AGG CCA GAC TTT GT-3'; *Hprt* probe, 5'-FAM-TTG CAG ATT CAA CTT GCG CTC A XT CTT-3'; *IDO* primers, 5'-GCT CGC AGT AGG GAA CAG CA-3', and 5'-CCT GGG TCC TTG TGG CTA GA-3'; *IDO* probe, 5'-FAM-TCg AGA AGA AGT TGA AAA GCT GCC CAC ACT XT A-3'.

The PCR results were obtained as exportable computer data. For each probe the amplification plots obtained and analyzed, cT is the cycle number at which the amplification reaction begins. Each probe was normalized to *HPRT* by calculating the difference between the cT for *HPRT* and the cT for *IDO* as follows:  $\Delta cT = cT \text{ HPRT} - cT \text{ IDO}$ . Because a lower  $\Delta cT$  means more cDNA that was obtained from RNA, and taking into account the exponential increase of cDNA in PCR, the initial mRNA quantity was calculated by using the formula described as follows:  $mRNA^* = 1^{**/2^{\Delta cT^{***}}}$  (\*, The source of the amplified cDNA was mRNA; \*\*, inverse ratio, because a lower  $\Delta cT$  means more DNA; and \*\*\*, the amplification product increases exponentially).

#### Statistical analysis

The InStat (Graph Pad Software) computer program was used. Data obtained by flow cytometry and abortion rate data were analyzed by the nonparametric Mann-Whitney *U* test. Asymmetric IgG molecule data were analyzed by Tukey's Multiple Comparisons Test. The mRNA levels, evaluated by real-time RT-PCR for *IDO*, normalized to *HPRT*, and represented as  $1/2^{\Delta cT}$ , were calculated for each sample, so that the mean and SEM were obtained for each group. The differences between group means were also evaluated using Kruskal-Wallis test, followed by Mann-Whitney *U* test. In all cases,  $p < 0.05$  was considered a statistically significant difference.

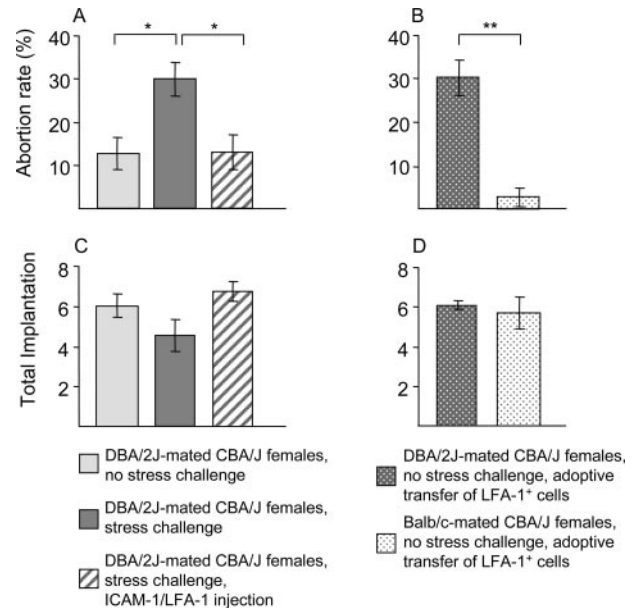
## Results

### LFA-1 and ICAM-1 expression are up-regulated in failing pregnancies challenged by stress

First, we were able to reproduce the increase in abortion upon stress exposure on Gd 5.5 without affecting the total number of implants, which confirmed the validity of the high abortion CBA/J  $\times$  DBA/2J mouse model (Fig. 1, A and C). This provided the necessary platform to address our key questions of the present study. We observed a high percentage of LFA-1<sup>+</sup> uterine cells in stressed mice, which reached levels of significance 4 days upon stress exposure, on Gd 10.5 (Fig. 2). Expression of ICAM-1 at the fetomaternal interface was assessed semiquantitatively by immunohistochemistry; representative examples for the respective groups are depicted in Fig. 3, A–D. As depicted in Table II, we observed a differential expression pattern with a high expression of ICAM-1 in stressed mice and a weak ICAM-1 expression in control mice in the three distinct areas investigated (endometrium, stroma, fetomaternal interface) on Gd 7.5, as well as on Gd 10.5.

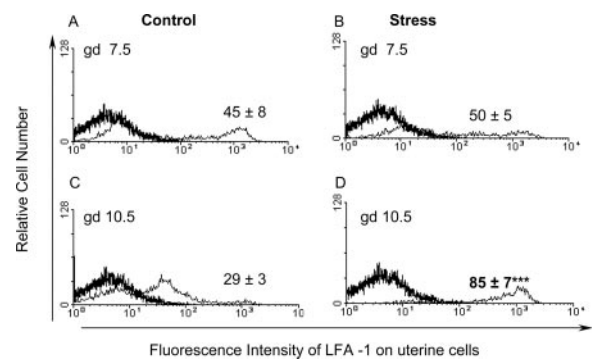
### Neutralization of cell adhesion molecules abrogates the detrimental effects of stress on the abortion rate, decidual Th1/Th2 ratio, and tolerance mechanisms (*IDO* expression, *Treg* cells, and AAbs)

As depicted in Fig. 1A, neutralization of adhesion molecules ICAM-1/LFA-1 significantly abolishes the effect of stress on the abortion rate. Next, we wished to determine the balance of Th1/

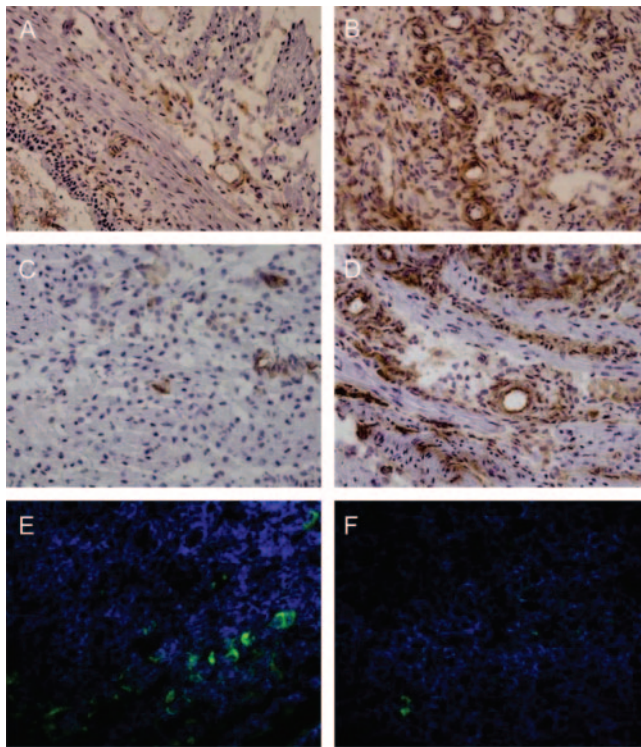


**FIGURE 1.** Stress exposure increases abortion rate in mice, which may be abrogated by ICAM-1/LFA-1 treatment and mimicked by adoptive transfer of LFA-1<sup>+</sup> cells. *A*, Stress exposure on Gd 5.5 challenges pregnancy maintenance and results in an increased abortion rate, as determined on Gd 13.5. Anti-ICAM-1/LFA-1 treatment during pregnancy abrogates the effect of stress challenge on the abortion rate. *B*, Adoptive transfer of LFA-1<sup>+</sup> blood cells into CBA/J increases the abortion rate exclusively in DBA/2J-mated CBA/J females, but not in BALB/c-mated CBA/J. The bars in *A* and *B* depict the mean percentage of abortion per group  $\pm$  SEM. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ , as analyzed by the nonparametric Mann-Whitney *U* test. *C* and *D* depict the mean number of implantations in each group, which does not significantly differ among the groups.

Th2 cytokines produced by subsets of uterine cells of our experimental groups, because this ratio has been proven to play an important role in the maintenance of pregnancy (3, 4, 29), and a Th2 cytokine pattern is involved in successful pregnancy outcome (28). We examined the intracellular expression of Th1 (TNF- $\alpha$ , IFN- $\gamma$ ,

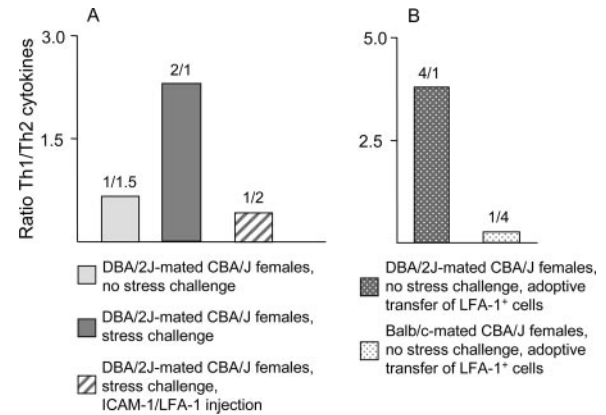


**FIGURE 2.** LFA-1 expression on uterine cells, as analyzed by flow cytometry. Histograms show representative examples of control and stress groups with uterine cells isolated on Gd 7.5 (*A* and *B*) and 10.5 (*C* and *D*). In the right corner of each plot, the mean of the percentage of cells positive for LFA-1  $\pm$  SEM is depicted; a significant increase of LFA-1<sup>+</sup> uterine cells could be observed on Gd 10.5, hence 4 days upon completion of stress challenge. \*\*\*,  $p < 0.001$  as calculated by nonparametric Mann-Whitney *U* test. Significance of difference was also calculated with respect to the experimental days, and we observed a significant decrease of LFA-1<sup>+</sup> uterine cells in control mice from Gd 7.5 to 10.5 ( $p < 0.01$ ), and an significant increase of LFA-1<sup>+</sup> uterine cells from stressed mice from Gd 7.5 to 10.5 ( $p < 0.01$ ).



**FIGURE 3.** ICAM-1 expression in pregnant uterus in the CBA/J × DBA/2J mouse model. *A* and *B* give representative examples of ICAM-1 immunohistochemistry staining on Gd 7.5 in control (*A*) and stressed mice (*B*). *C* and *D* show ICAM-1 staining of tissue obtained on Gd 10.5; *C* reflects staining from control mice, and *D* shows representative staining of uteri from stressed mice, where exposure to stress increases the ICAM-1 expression in endometrium, stromal cells, and at the fetomaternal interface. All tissues have been counterstained with Meyer's hematoxylin for better orientation. *E* and *F*, Immunofluorescence of LFA-1<sup>+</sup> cells in the uterus. *E*, To confirm the migration of injected LFA-1<sup>+</sup> cells from blood into uterus, the LFA-1<sup>+</sup> cells were labeled with CFSE<sup>+</sup> fluorescent dye before injection. Subsequently, the presence of the CFSE<sup>+</sup> dye could be detected at the fetomaternal interface (green cells) in DBA/2J-mated CBA/J females, which delivers the proof that these cells indeed migrated from the blood into the uterus. *F* shows a representative example on an uterus from the low abortion mouse model of BALB/c-mated CBA/J females injected with LFA-1<sup>+</sup>/CFSE<sup>+</sup> cells, where very few cells could be detected. Magnification in *A–D* is ×200, and in *E* and *F* is ×400.

and IL-12) and Th2 (IL-4 and IL-10) cytokines in uterine cells during the crucial stage of early pregnancy, the peri-implantation period on Gd 7.5. As depicted in Fig. 4*A*, we observed that the ratio was increased in favor of Th1 in stressed mice, which could be abrogated by neutralization of ICAM-1/LFA-1.



**FIGURE 4.** Th1/Th2 cytokine ratio in uterine cells is modified by treatment with mAb against ICAM-1/LFA-1 and adoptive transfer of LFA-1<sup>+</sup> cells. Th1/Th2 ratio has been calculated by adding the percentage of TNF- $\alpha$ <sup>+</sup>, IFN- $\gamma$ <sup>+</sup>, and IL-12<sup>+</sup> cells, and this sum was then divided by 3 to obtain the Th1 value. The percentages of IL-4<sup>+</sup> and IL-10<sup>+</sup> uterine cells were also added, and this sum was divided by 2 to obtain the Th2 value. The ratio Th1/Th2 was obtained by dividing the Th1 value by the Th2 value. Exact ratios are indicated on top of each bar. *A*, An increased Th1/Th2 ratio could be observed in mice challenged by stress, which could be abrogated by anti-ICAM-1/LFA-1 injection. *B*, An increased Th1/Th2 ratio could be induced by adoptive transfer of LFA-1<sup>+</sup> cells exclusively in DBA/2J-mated CBA/J females, but not in BALB/c-mated CBA/J.

As previously mentioned, the fetus represents a foreign entity to the maternal immune system, yet this “natural” allograft is generally not rejected, which has been suggested to be attributable to plural tolerance mechanisms. One of them is the expression of IDO, which catalyzes the initial and rate-limiting step in the metabolism of tryptophan along the kynurenine pathway in mammals and hereby deprives T cells and inhibits lymphocyte proliferation (6–8). We quantified the expression of IDO mRNA in uterine tissue from pregnant mice on day 7.5 of gestation and observed that stressed CBA/J female mice expressed significantly lower mRNA of IDO compared with ICAM-1/LFA-1-neutralized, stressed CBA/J females (Fig. 5*A*).

Following recently published data on CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells essential for tolerance, e.g., against the fetus (9, 10, 30, 31), we investigated the percentage of this distinct subpopulation within the uterine population. As depicted in Fig. 5*C*, stressed mice presented a significantly lower percentage of CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells in comparison with nonstressed, control, and anti-adhesion molecule-treated, stressed mice.

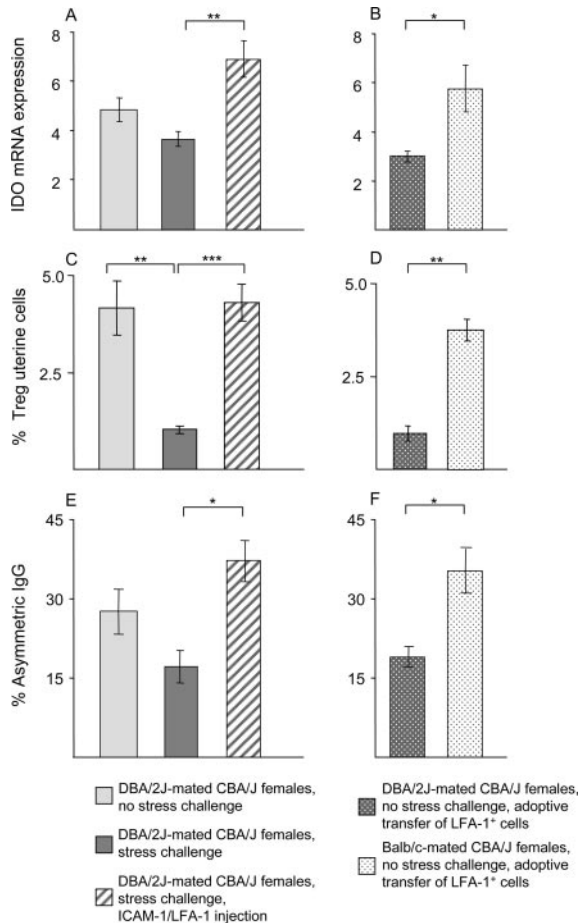
The third tolerance mechanism with relevance for pregnancy maintenance we investigated was the percentage of AAbs among

Table II. ICAM-1 expression in uteri of high and low abortion pregnancies

Group	Mated to	Gd	Transfer <sup>a</sup>	Epithelium <sup>b</sup>	Stroma <sup>b</sup>	Feto-maternal Interface <sup>b</sup>
Control	DBA/2J	7.5	–	+	+	+
Stress	DBA/2J	7.5	–	+++	+++	+++
Control	DBA/2J	10.5	–	–/+	+	+
Stress	DBA/2J	10.5	–	+++	+++	+++
Control	DBA/2J	7.5	+	+++	+++	+++
Control	BALB/c	7.5	+	+	+	–/+

<sup>a</sup> Transfer of LFA-1<sup>+</sup> blood cells from stressed mice on Gd 6.5, – means groups received no transfer, and + means that groups received the LFA-1 transfer i.v.

<sup>b</sup> The intensity of staining signals in distinct structural components of the uterus (epithelium, stroma, fetomaternal interface) was scored as follows: –, negative; –/+, patchy; +, weak; ++, moderate; +++, high.



**FIGURE 5.** Immune tolerance mechanisms (IDO expression,  $CD4^+ CD25^{\text{bright}}$  uterine cells, and percentage of AAbs) are modified by treatment with mAb against ICAM-1/LFA-1 and adoptive transfer of LFA-1 $^+$  cells. *A* and *B*, mRNA expression of uterine IDO, as quantified by real-time PCR. IDO expression was reduced in uteri from matings challenged by stress, which could be significantly prevented by injection of anti-ICAM-1/LFA-1. *B*, Adoptive transfer of LFA-1 $^+$  cells resulted in low uterine IDO mRNA expression in CBA/J  $\times$  DBA/2J matings, but not in CBA/J  $\times$  BALB/c mice. *C* and *D*, Percentages of  $CD4^+ CD25^{\text{bright}}$  uterine cells of the experimental groups on Gd 7.5. Data are represented as the mean  $\pm$  SEM, and significance of differences was calculated by the nonparametric Mann-Whitney *U* test. *E* and *F*, Percentages of AAbs in serum from pregnant mice on Gd 7.5. Results are expressed as mean  $\pm$  SEM. Tukey's multiple comparison test was used to determine differences between the groups. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; and \*\*\*,  $p < 0.001$ .

the entire IgGs, because AAbs have been suggested to mediate pregnancy protection by camouflaging paternal Ags (11–13). As shown in Fig. 5*E*, ICAM-1/LFA-1 neutralization augmented the

percentage of AAbs on Gd 7.5 in stressed mice, compared with the stress group.

Because a strong focus in successful reproduction is on the ratio of Th1/Th2 cytokines in the decidua, we correlated the individual data per mouse on the Th1/Th2 ratio presented in Fig. 4 to the ICAM-1 score presented in Table II (after the “+” have been transformed in arbitrary units) and obtained a positive linear regression of  $r^2 = 0.98$ . Furthermore, the Th1/Th2 ratio was negatively correlated to 1) the percentage of AAb ( $r^2 = 0.78$ ), 2) the decidua IDO mRNA expression ( $r^2 = 0.76$ ), and 3) Treg cells ( $r^2 = 0.97$ ). However, because correlation does not imply causality, further studies are required to investigate whether ICAM-1/LFA-1, IDO expression, synthesis of AAb, and presence of Treg are under the direct regulation of cytokines or vice versa.

*Increased migration of different leukocyte subsets into decidual tissue in pathological pregnancies challenged by stress may be abrogated by blocking ICAM-1/LFA-1*

To elucidate whether ICAM-1/LFA-1 pathways are involved in an increased migration of different leukocyte subsets into decidual tissue, which may then be detrimental for pregnancy maintenance, we analyzed the phenotype of leukocyte subsets in our experimental groups. As shown in Table III, the percentage of NK1.1, F4/80 (macrophages),  $CD4^+ CD25^-$ , and TCR- $\gamma\delta$  cells among the uterine lymphocyte population was restored to levels seen in non-stressed control mice; hence the increased migration in pathological pregnancies upon stress exposure in CBA/J  $\times$  DBA/2J matings could be abrogated by blocking ICAM-1/LFA-1.

*ICAM-1/LFA-1 affects maturity, Th1 predominance, and lineage of dendritic uterine cells*

Pioneering work of Kämmerer et al. (14) showed that DC are a population with special regulatory functions at the feto-maternal interface, whereby it is crucial to differentiate between distinct subsets of mammalian DCs, e.g., lymphoid/myeloid, immature/mature, or Th1/Th2 phenotype (16). In the present work, we optimized the phenotypical evaluation of DCs by using a CD11c-enriched population, which allows a clear differentiation of DC subsets within the CD11c $^+$  cell population by subsequent flow cytometry. Our previously published data indicate that the proportion of CD11c $^+$  cells among the uterine cell population is ~10–15% (16). As shown in Table IV, uterine DCs from pathological pregnancies as seen in stressed DBA/2J-mated CBA/J female mice clearly displayed an increased positivity for CD8 $\alpha$  (sign for lymphoid lineage and maturity), CD80 (costimulatory molecule/sign for maturity), and MHC-II (sign for maturity), and an increased ratio of Th1/Th2 (IL-12 > IL-10) cytokines in comparison with undisturbed pregnancies. Interestingly, blocking of ICAM-1/LFA-1 abrogated the effect of stress challenge on uterine DC phenotype.

Table III. *The percentage of leukocyte subsets from uterine cells*

Group	Mated to	mAbs <sup>a</sup>	Transfer <sup>b</sup>	F4/80	NK1.1	TCR- $\gamma\delta$	$CD4^+ CD25^-$
Control	DBA/2J	–	–	5.9 $\pm$ 0.9	6.8 $\pm$ 0.8	8.2 $\pm$ 0.2	6.4 $\pm$ 0.4
Stress <sup>c</sup>	DBA/2J	–	–	11.0 $\pm$ 1.4	<b>13.7 <math>\pm</math> 1.5</b>	<b>16.2 <math>\pm</math> 0.4</b>	9.8 $\pm$ 1.3
Stress	DBA/2J	+	–	7.0 $\pm$ 0.7	7.1 $\pm$ 1.0	10.8 $\pm$ 1.3	6.6 $\pm$ 0.6
Control <sup>d</sup>	DBA/2J	–	+	<b>18.0 <math>\pm</math> 1.5</b>	<b>14.0 <math>\pm</math> 0.3</b>	<b>17.0 <math>\pm</math> 1.0</b>	<b>14.4 <math>\pm</math> 1.8</b>
Control	BALB/c	–	+	7.6 $\pm$ 1.5	6.5 $\pm$ 1.0	7.4 $\pm$ 0.8	5.8 $\pm$ 0.4

<sup>a</sup> Treatment with mAbs against ICAM-1 and LFA-1. – equals no injection of mAbs, and + represents injection of mAbs.

<sup>b</sup> Transfer of LFA-1 $^+$  blood cells from stressed mice on Gd 6.5. – means groups received no transfer, and + means that groups received the LFA-1 transfer i.v.

<sup>c</sup> Numbers in boldface depict statistically significant differences of anti-adhesion molecules treated and control mice to stressed.

<sup>d</sup> Percentages in boldface are significantly different in CBA/J  $\times$  DBA/2J matings, compared with CBA/J  $\times$  BALB/c matings.



Table IV. Phenotype and lineage of uterine DCs upon enrichment for CD11c<sup>+</sup>

Group	Mated to	mAbs <sup>a</sup>	Transfer <sup>b</sup>	Myeloid (CD8α <sup>+</sup> )	Lymphoid (CD8α <sup>+</sup> )	MHC-II <sup>+</sup> /MCH-II <sup>c</sup>	CD80 <sup>+</sup>	Th1/Th2 <sup>c</sup>
Control	DBA/2J	–	–	99.3 ± 1.9	0.7 ± 0.1	0.6	18.2 ± 1.5	1/2.5
Stress <sup>d</sup>	DBA/2J	–	–	96.4 ± 0.9	<b>3.6 ± 0.2</b>	0.9	<b>33.4 ± 2.0</b>	<b>2/1</b>
Stress	DBA/2J	+	–	99.2 ± 0.7	0.8 ± 0.1	0.6	21.6 ± 3.8	1/3
Control <sup>e</sup>	DBA/2J	–	+	94.1 ± 0.5	<b>5.9 ± 0.4</b>	1.3	<b>32.4 ± 2.7</b>	<b>3.5/1</b>
Control	BALB/c	–	+	99.3 ± 0.6	0.7 ± 0.3	0.7	20.2 ± 1.0	1/10

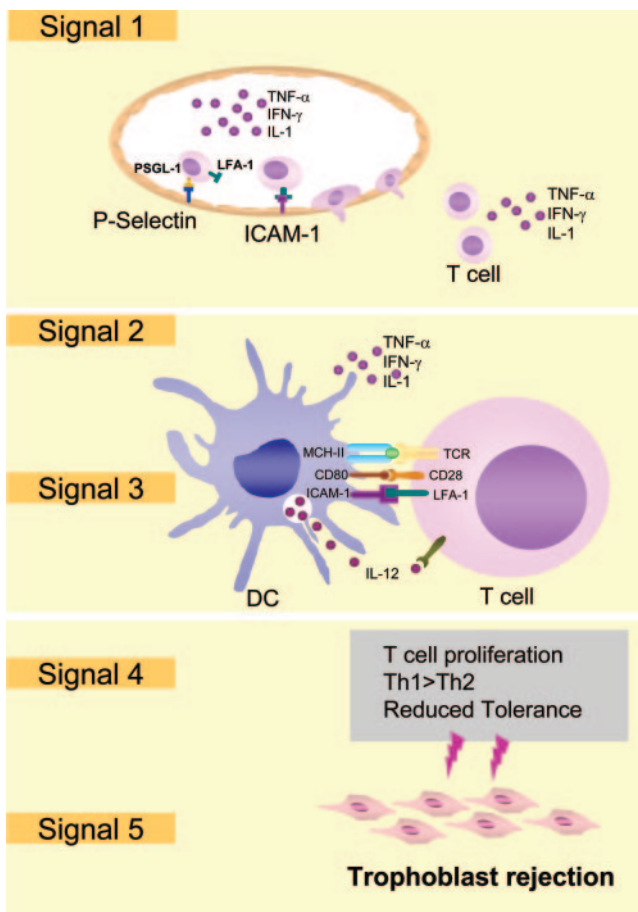
<sup>a</sup> Treatment with mAbs against ICAM-1 and LFA-1. – equals no injection of mAbs, and + represents injection of mAbs.

<sup>b</sup> Transfer of LFA-1<sup>+</sup> blood cells from stressed mice on Gd 6.5. – means groups received no transfer, and + means that groups received the LFA-1 transfer i.v.

<sup>c</sup> Ratio of MCH-II<sup>+</sup>/MCH-II<sup>–</sup> has been calculated dividing the percentage of CD11c<sup>+</sup>/MCH-II<sup>+</sup> to the percentage of CD11c<sup>+</sup>/MCH-II<sup>–</sup>. Th1/Th2 cytokines ratio has been obtained by division of percentage of CD11c<sup>+</sup>/IL-12 to the CD11c<sup>+</sup>/IL-10.

<sup>d</sup> Significant differences of percentages of CD8α<sup>+</sup>, CD80<sup>+</sup>, and Th1/Th2 ratio from stressed mice compared to control and anti-adhesion molecule-treated mice are presented in boldface.

<sup>e</sup> Percentages of CD11c<sup>+</sup>/CD8α<sup>+</sup> and CD11c<sup>+</sup>/CD80<sup>+</sup> cells and ratio of MHC-II<sup>+</sup>/MCH-II<sup>–</sup> and Th1/Th2 from transferred CBA/J × DBA/2J mice are significantly higher, compared with CBA/J × BALB/c mice.



**FIGURE 6.** Hypothetical scenario of our understanding why a woman's immune system may reject her histoincompatible fetus in challenged pregnancies. Cross talk between ICAM-1, an adhesion molecule expressed on epithelium, endothelium, and APC, and its ligand, LFA-1, leads to increased recruitment of abortogenic Th1 cytokine-expressing cells to the implantation site in challenged, abortion-prone pregnancies (signal 1) and additional Th1 polarization and clonal expansion via mature DCs (signals 2 and 3). Subsequently, fetal tolerance, e.g., IDO expression, presence of CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells, and synthesis of AABs are disrupted (signal 4). Hence, our data shed light on a hierarchical network of immune integration at the feto-maternal interface, in which ICAM-1/LFA-1 cross talk is clearly a proximate mediator capable of disrupting successful pregnancy maintenance by inducing trophoblast rejection (signal 5).

### Transfer of LFA-1<sup>+</sup> cell mimics stress effects exclusively in abortion-prone matings

To address the question whether LFA-1<sup>+</sup> blood cell transfer would suffice to mimic the effects of stress in abortion-prone mating combinations, as well as in low abortion mating combinations, we injected DBA/2J- or BALB/c-mated CBA/J females with LFA-1<sup>+</sup> blood cells obtained from stressed, DBA/2J-mated CBA/J females. Transferred LFA-1<sup>+</sup> cells, which included high numbers of CD3 cells and CD8α<sup>+</sup> cells, and expressed high levels of Th1 cytokines, provoked an increase of the abortion rate in CBA/J × DBA/2J high abortion mouse model (Fig. 1B); again, the number of implantations did not differ between the groups (Fig. 1D). We confirmed the migration of the injected cell population from blood into uterus by labeling the LFA-1<sup>+</sup> cells with CFSE<sup>+</sup> fluorescent dye, which could subsequently be detected at the feto-maternal interface, and hence provided convincing evidence that these cells indeed migrated from the blood into the uterus and may consecutively mediate fetal demise (Fig. 3E). Interestingly, the low abortion mouse model of BALB/c-mated CBA/J females presented a very low abortion rate upon injection with LFA-1<sup>+</sup> cells, and only very few cells could be detected in the uterus (Fig. 3F). Furthermore, expression of ICAM-1 in pregnant uteri were exclusively up-regulated in DBA/2J-mated females, but not in BALB/c-mated females injected with LFA-1<sup>+</sup> cells, as shown in Table II.

Moreover, LFA-1<sup>+</sup> cell transfer into CBA/J females induced an increase of the Th1/Th2 ratio, and reduced tolerance mechanisms (IDO expression, CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells, and AABs; Fig. 5, A–C, right) in DBA/2J-mated mice, but not in BALB/c-mated mice (B, D, and F). A dramatic decline of NK, TCR-γδ, CD4<sup>+</sup>CD25<sup>–</sup> cells, and F4/80<sup>+</sup> (macrophages) was clearly manifested in CBA/J × BALB/c pregnant mice injected with LFA-1<sup>+</sup> cells in comparison with CBA/J × DBA/2J (Table III). In addition, CBA/J × DBA/2J mice injected with LFA-1<sup>+</sup> cells show an increase of DC expressing CD8α, MHC-II, and CD80, and an increased ratio of Th1/Th2 cytokines, compared with CBA/J × BALB/c mice (Table IV).

## Discussion

In this study, we deliver considerable evidence for a functional role of ICAM-1 and LFA-1 during the peri-implantation period in mammalian pregnancies by demonstrating that pregnancy maintenance may be severely affected via this distinct adhesion molecule-dependent pathway. These observations constitute a central finding of our work, motivating us to discuss the following scenario depicted in Fig. 6.



### *Selective recruitment of leukocytes into the decidua is facilitated*

High uterine expression of ICAM-1 in epithelium as well as in stroma of abortion-prone matings, possibly additionally triggered by an environmental challenge, e.g., high stress perception, results in an increased recruitment of lymphocytes expressing LFA-1 from the blood into the uterus (signal 1). Cytokines such as IL-1, TNF- $\alpha$ , and IFN- $\gamma$ , which are increased in blood lymphocytes upon stress exposure, are known to further increase the endothelial expression of ICAM-1 (18), which provides an explanation for the selective recruitment of leukocytes into the murine decidua. This also supports our previous observation indicating that Th1 lymphocyte migration into decidua is enhanced in spontaneous abortions in humans, due to up-regulated expression of adhesion molecules; we formerly investigated P selectin-dependent pathways (32). Interestingly, in our present work in mice, we were also able to detect an increase of P selectin and its corresponding ligand, P-selectin glycoprotein ligand 1 (data not shown).

Noteworthy, blastocyst adherence and subsequent trophoblast invasion induces vigorous changes in cell-cell and cell matrix interaction and initially causes a physiological Th1 reaction in the developing decidua in undisturbed pregnancies. It has been suggested that this early Th1 predominance is required to restrict inner cell mass proliferation in the blastocyst and affects the ratio of mononucleated to multinucleated trophoblast cells (33). This is supported by our present data, where we observe a rather high percentage of LFA-1<sup>+</sup> decidual cells in control mice during early gestation (Gd 7.5), which is significantly reduced upon completion of implantation (Gd 10.5).

### *Increased decidual Th1 cytokines induce maturation of APC by up-regulation of ICAM-1*

ICAM-1/LFA-1 interaction has pleiotropic effects, because it not only plays an important role in the T cell recruitment but also promotes cross talk between APC and T cells (34). APC constitute a complex system of cells, which, under different microenvironmental conditions, can induce such contrasting states as immunity or tolerance. Immature DCs have been described to reside in early pregnancy decidua in humans (14, 15) and mice (16), and possibly serve as sentinel cells of the tissue environment for potential danger signals. Numerous factors induce and/or regulate DCs maturation via TLR, including the balance between Th1 and Th2 signals in the local microenvironment; such maturation provides the ability of DCs to become mature APC and initiate adaptive immune responses (17). During gestation in mice, ~25% of all DCs are mature, and—strikingly—when implantation occurs (Gd 5.5–8.5), this percentage is markedly down-regulated in low abortion pregnancies (16). However, in pregnancies with high abortion rates, e.g., as induced by stress challenge or adoptive transfer of LFA-1 cells, we observe an increase of mature DCs, as identified by their CD8 $\alpha$ , CD80, and MHC-II positivity.

During the maturation process, DCs strongly up-regulate costimulatory molecules, e.g., CD80 and ICAM-1, which is depicted as signal 2 in Fig. 6. Noteworthy, recognition of MHC-peptide complexes on DCs with the TCR provides the prerequisite for APC-T cell clustering and subsequent cytokine polarization (17). However, the crucial factor required to sustain T cell activation is the interaction between costimulatory molecules, e.g., CD80 and ICAM-1 expressed by APC, and their ligands, e.g., CD28 and LFA-1, expressed by T cells (18, 34). Markedly, a high density of TCR-MHC-Ag complexes cannot compensate for the lack of a costimulatory signal such as ICAM-1/LFA-1 interaction, indicating the importance of the ICAM-1/LFA-1 costimulatory signaling pathway (17). This secondary signal can influence the type of re-

sponse generated by T cells; thus DC subsets may provide T cells with the different cytokine/molecule microenvironments that determine the classes of immune response, for example, Th1 vs Th2 or Treg cytokine profile. Several mouse studies have revealed that the ICAM-1/LFA-1 interaction appears to be important for polarizing cells toward Th1 predominance (signal 3 in Fig. 6); most prominent in this respect is DC-derived IL-12 (34–36), which we observed to be increased in high abortion matings, but not in low abortion matings. Intriguingly, such Th1/Th2 skewing may apply for CD4 T cells, as well as CD8 T lymphocytes and NK T cells (36).

DCs may exhibit considerable plasticity in their ability to induce temporal regulation of Th responses, and DCs that normally induce Th1 profiles can be converted to Th2-skewing cells when treated with Th2 cytokines or in response to steroids, e.g., glucocorticoids and possibly progesterone (37). This is particularly interesting because we previously reported decreased progesterone levels in mice in response to stress (38), which subsequently led to a predominance of Th1 cytokine production (28), suggesting that progesterone is a prerequisite for a DC-induced Th2 predominance. Furthermore, the conversion of DC into tolerogenic cells has recently been reported to be mediated by decrease of ICAM-1, CD80, and CD86 (39).

### *Clonal expansion and reduced tolerance in response to ICAM-1/LFA-1 binding*

The ICAM-1/LFA-1 binding and subsequent Th1 skewing of CD4 T cells, CD8 T cells, and/or NK T cells may further result in clonal expansion of such population in various inflammatory settings, as indicated by our present findings and others (37, 40, 41), which may then additionally attribute to a sustained Th1 predominance, as pointed out as signal 4 in Fig. 6. Furthermore, we observed reduced tolerance, as identified by decreased expression of the tryptophan-catabolizing enzyme IDO, decreased percentage of Treg cells and AAbs (signal 4 in Fig. 6).

Immature murine DCs are capable of suppressing T cell responses and promote tolerance, rather than initiate adaptive immunity (42–44). Expression of IDO on such DC subsets may suppress T cell response to fetal alloantigens during normally progressing murine pregnancy (6, 45). Recent findings indicate that CTLA4-Ig induces IDO expression on specific DC subsets (7, 46). Based on the available literature and our current findings, we propose that the ICAM-1/LFA-1-mediated disequilibrium of immature to mature DCs in high abortion pregnancies results in reduced expression of IL-10 and also IDO.

An additional mechanism of tolerance, the presence of CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells, has been investigated in the present study; these cells were reduced in high abortion pregnancies. Recently, it has been reported that Treg cells are required for the maternal immune system to tolerate the fetal allograft (9, 10). This clearly supports our current data, suggesting that decreased CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells might induce maternal lymphocyte activation to reject the fetal allograft. Recent findings point out that CD4<sup>+</sup>CD25<sup>bright</sup> Treg inhibit the proliferation of autologous CD4<sup>+</sup>CD25<sup>-</sup> T cells (10). This is in line with our observation of a high percentage of CD4<sup>+</sup>CD25<sup>-</sup> uterine cells in high abortion pregnancies accompanied by low numbers of CD4<sup>+</sup>CD25<sup>bright</sup> cells. Immature mucosal, IL-10-producing DCs may favor the induction of Th2 or Treg cells (47, 48), which again points toward a significance of pregnancy-protective immature-tolerogenic DCs in pregnancy maintenance. In contrast, a Th1-induced maturation of DCs suppresses tolerance mechanisms and subsequently results in pregnancy failure.

An additional mechanism favoring the immunological symbiosis between mother and fetus is the production of AAbs (11–13). These AAbs are unable to activate effector mechanisms of the immune response. However, AAbs may block effector cell targets, e.g., paternal Ags expressed by the placenta (12, 49). We hypothesize that the ICAM-1/LFA-1-mediated Th1 > Th2 skew results in a decreased AAb synthesis by B cells. Hence, fetal Ags may not be camouflaged and fail to escape NK and/or T cell-mediated maternal attack.

#### *Damage of fetal tissue by migrating LFA-1<sup>+</sup> cells*

Our data do not conclusively prove that LFA-1<sup>+</sup> cells migrate to the placenta region and damage fetal tissue. However, others have shown that maternal LFA-1<sup>+</sup> cells bind to the placental barrier and subsequently induce focal damage mediated by Th1 cytokines (50). Furthermore, HIV-1 vertical transmission in utero appears to be mediated by LFA-1-mediated cell-to-cell contact (51). Moreover, maternal T cells may also bind to trophoblast ICAM-1 via activated LFA-1 and hereby provoke apoptosis (52). Taken together, these observations strongly suggest a LFA-1/ICAM-1-dependent route for maternal leukocyte migration to the placental barrier with subsequent tissue damage.

#### *Transfer of LFA<sup>+</sup> cell mimics stress effects exclusively in abortion-prone matings*

In our present work, we have used two murine mating combinations, CBA/J females mated to either DBA/2J or BALB/c males. Thus, as the fetal trophoblasts of either mating combination invade the uterine wall at implantation, circulating blood and/or resident decidual immune cells may be exposed to fetal Ags derived from DBA/2J or BALB/c males. In both mating combinations, fetal losses were rather low in control mice. Strikingly, upon an eliciting stimulus such as adoptive transfer of LFA-1<sup>+</sup> cells, the abortion rate increased exclusively in DBA/J-mated, but not in BALB/c-mated CBA/J. This allows the hypothesis that the fetus itself, or—more precisely—Ags expressed on fetal cells, modulate the nature and constituents of the maternal immune response. This hypothesis is supported by observations of NK and NKT cell infiltrates at the fetomaternal interface in CBA/J × DBA/2J matings, beginning within 2 days of implantation (2, 53), which may be facilitated by the high ICAM-1 expression we observed exclusively in DBA/2J-mated females. Furthermore, a protective CD8 T cell population present in CBA/J × BALB/c matings or unchallenged CBA/J × DBA/2J matings may prevent an NK cell-mediated high abortion rate (28, 54). Therefore, we emphasize that distinct characteristics of paternal Ag expression are crucial for the fate of the conceptus. This may be translated from bench to bedside, but also from bedside to bench, e.g., frequent reports of women suffering from pregnancy complications may have an uncomplicated subsequent pregnancy with another partner (55, 56). Because our current understanding of Ag expressed by murine trophoblasts in normal and abnormal pregnancies is still incomplete (57, 58), additional research is required to recognize the nature and constituents of the ensuing maternal immune response.

#### *Rejection of the trophoblast as a consequence of immune disintegration*

We propose that trophoblast rejection is a consequence of an immune disintegration, which includes ICAM-1/LFA-1 mediated recruitment of Th1 cells to the fetomaternal interface and additional Th1 polarization of decidual immune cells, reduced tolerance, and endocrine dysbalances, such as insufficient levels of progesterone. In summary, nothing in reproductive immunology is as simple as it first appears. A Th1 > Th2 predominance as a sole explanation

for pregnancy failure—as propagated by many publications—seems clearly and exceedingly crude, and the so-called Th1/Th2 paradigm should be seen as part of hierarchical network, in which endocrine and multiple immune signals need to be integrated. We propose that diverse cell populations, such as immature tolerogenic IL-10<sup>+</sup> DCs, CD4<sup>+</sup>CD25<sup>bright</sup> Treg cells, and B cells producing AAbs are functionally contributing to pregnancy maintenance. Within this hierarchical network, ICAM-1/LFA-1 cross talk is clearly a proximate mediator capable of disrupting the collective effort of immune integration at the fetomaternal interface, hereby threatening successful pregnancy maintenance.

As the understanding of ICAM-1/LFA-1 interaction has grown in tumor or transplantation immunology, so too has the pharmaceutical industry's interest in developing ways to modulate this interaction. The therapeutic effectiveness of inhibiting ICAM-1/LFA-1 binding are currently being investigated in multiple diseases, some of which have already reached phase III trials (59–61), and prevention of pregnancy failure may also become an indication for such intervention.

## Acknowledgments

We are grateful for the excellent technical assistance of the members of the PsychoNeuroImmunology Laboratory ([www.pni-labor.de](http://www.pni-labor.de)) and appreciate the effort of Margarita Strozynski (Department of Pediatrics, Charité) for sharing her expertise in i.v. injections with us.

## References

- Norwitz, E. R., D. J. Schust, and S. J. Fisher. 2001. Implantation and the survival of early pregnancy. *N. Engl. J. Med.* 345:1400.
- Clark, D. A., P. C. Arck, and G. Chaouat. 1999. Why did your mother reject you? Immunogenetic determinants of the response to environmental selective pressure expressed at the uterine level. *Am. J. Reprod. Immunol.* 41:5.
- Lin, H., T. R. Mossmann, L. Guilbert, S. Tuntipopipat, and T. G. Wegmann. 1993. Synthesis of T helper 2-type cytokines at the fetomaternal interface. *J. Immunol.* 151:4562.
- Piccini, M. P., L. Beloni, C. Livi, E. Maggi, G. Scarselli, and S. Romagnani. 1998. Defective production of both, leukemia inhibitory factor and type 2-T helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat. Med.* 4:1020.
- Joachim, R. A., M. Hildebrandt, J. Oder, B. F. Klapp, and P. C. Arck. 2001. Murine stress-triggered abortion is mediated by increase of CD8<sup>+</sup>TNF- $\alpha$ <sup>+</sup> decidual cells via substance P. *Am. J. Reprod. Immunol.* 45:303.
- Munn, D. H., M. Zhou, J. T. Attwood, I. Bondarev, S. J. Conway, B. Marshall, C. Brown, and A. L. Mellor. 1998. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281:1191.
- Munn, D. H., M. D. Sharma, J. R. Lee, K. G. Jhaver, T. S. Johnson, D. B. Keskin, B. Marshall, P. Chandler, S. J. Antonia, R. Burgess, et al. 2002. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* 297:1867.
- Terness, P., T. M. Bauer, L. Rose, C. Dufter, A. Watzlik, H. Simon, and G. Opelz. 2002. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J. Exp. Med.* 19:447.
- Sasaki, Y., M. Sakai, S. Miyazaki, S. Higuma, A. Shiozaki, and S. Saito. 2004. Decidual and peripheral blood CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol. Hum. Reprod.* 10:347.
- Aluvihare, V. R., M. Kallikourdis, and A. G. Betz. 2004. Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* 5:266.
- Malan Borel, I., T. Gentile, J. Angelucci, J. Pividori, M. C. Guala, R. A. Binaghi, and R. A. Margni. 1991. IgG asymmetric molecules with anti-paternal activity isolated from sera and placenta of pregnant human. *J. Reprod. Immunol.* 20:129.
- Margni, R. A., and I. Malan Borel. 1998. Paradoxical behavior of asymmetric IgG antibodies. *Immunol. Rev.* 163:77.
- Margni, R. A., and A. C. Zenclussen. 2001. During pregnancy, in the context of a Th2 type cytokine profile, serum IL-6 levels might condition the quality of the synthesized antibodies. *Am. J. Reprod. Immunol.* 46:181.
- Kämmerer, U., M. Schoppet, A. D. McLellan, M. Kapp, H.-I. Huppertz, E. Kämpgen, and J. Dietl. 2000. Human decidua contains potent immunostimulatory CD83<sup>+</sup> dendritic cells. *Am. J. Pathol.* 157:159.
- Gardner, L., and A. Moffett. 2003. Dendritic cells in the human decidua. *Biol. Reprod.* 69:1438.
- Blois, S. M., C. D. Alba Soto, M. Tometten, B. F. Klapp, R. A. Margni, and P. C. Arck. 2004. Lineage, maturity, and phenotype of uterine murine dendritic cells throughout gestation indicate a protective role in maintaining pregnancy. *Biol. Reprod.* 70:1018.
- Anderson, M. E., and T. J. Sahaan. 2003. Targeting ICAM-1/LFA-1 interaction for controlling autoimmune diseases: designing peptide and small molecule inhibitors. *Peptides* 24:487.

18. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76:301.
19. Dedrick, R. L., S. Bodary, and M. R. Garovoy. 2003. Adhesion molecules as therapeutic targets for autoimmune diseases and transplant rejection. *Expert Opin. Biol. Ther.* 3:85.
20. Takeshita, T., M. Satomi, S. Akira, Y. Nakagawa, H. Takahashi, and T. Araki. 2000. Preventive effect of monoclonal antibodies to intercellular adhesion molecule-1 and leukocyte function-associate antigen-1 on murine spontaneous fetal resorption. *Am. J. Reprod. Immunol.* 43:180.
21. Harning, R., J. Pelletier, K. Lubbe, F. Takei, and V. J. Merluzzi. 1991. Reduction in the severity of graft-versus-host disease and increased survival in allogeneic mice by treatment with monoclonal antibodies to cell adhesion antigens LFA-1 and MALA-2. *Transplantation* 52:842.
22. van Kooyk, Y., A. de Vries-van der Zwan, L. P. de Waal, and C. G. Figdor. 1994. Efficiency of antibodies directed against adhesion molecules to prolong skin graft survival in mice. *Transplant. Proc.* 26:401.
23. Kootstra, C. J., D. M. van der Giezen, J. H. J. M. van Krieken, E. de Heer, and J. A. Bruijn. 1997. Effective treatment of experimental lupus nephritis by combined administration of anti-CD11a and anti-CD54 antibodies. *Clin. Exp. Immunol.* 108:324.
24. Saban, M. R., R. Saban, D. Bjorling, and M. Haak-Frendscho. 1997. Involvement of leukotrienes, TNF- $\alpha$ , and the LFA-1/ICAM-1 interaction in substance P-induced granulocyte infiltration. *J. Leukocyte Biol.* 61:445.
25. Arck, P. C., F. S. Merali, J. Manuel, G. Chaouat, and D. A. Clark. 1995. Stress-triggered abortion: inhibition of protective suppression and promotion of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) release as a mechanism triggering resorptions in mice. *Am. J. Reprod. Immunol.* 33:74.
26. Scheynius, A., R. L. Camp, and E. Puré. 1993. Reduced contact sensitivity reactions in mice treated with monoclonal antibodies to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J. Immunol.* 150:655.
27. Masten, B. J., J. L. Yates, A. M. P. Koga, and M. F. Lipscomb. 1997. Characterization of accessory molecules in murine lung dendritic cell function: roles for CD80, CD86, CD54, and CD40L. *Am. J. Respir. Cell Mol. Biol.* 16:335.
28. Blois, S. M., R. Joachim, J. Kandil, R. Margni, M. Tometten, B. F. Klapp, and P. C. Arck. 2004. Depletion of CD8 $^{+}$  cells abolishes the pregnancy protective effect of progesterone substitution with hydrocortisone in mice by altering the TH1/TH2 cytokine profile. *J. Immunol.* 172:5893.
29. Raghupathy, R. 2001. Pregnancy: success and failure within the Th1/Th2/Th3 paradigm. *Semin. Immunol.* 13:219.
30. Kuniyasu, Y., T. Takahashi, M. Itoh, J. Shimizu, G. Toda, and S. Sakaguchi. 2000. Naturally anergic and suppressive CD25 $^{+}$ CD4 $^{+}$  T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. *Int. Immunol.* 12:1145.
31. Sakaguchi, S., N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda, and T. Takahashi. 2001. Immunologic tolerance maintained by CD25 $^{+}$ CD4 $^{+}$  regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev.* 182:18.
32. Zenclussen, A. C., S. Fest, U. S. Sehmsdorf, E. Hagen, B. F. Klapp, and P. C. Arck. 2001. Up-regulation of decidual P-selectin expression is associated with an increased number of Th1 cell populations in patients suffering from spontaneous abortions. *Cell. Immunol.* 213:94.
33. Whiteside, E. J., K. J. Boucalt, A. The, J. Garcia-Aragon, M. B. Harvey, and A. C. Herington. 2003. Elevated concentration of TNF- $\alpha$  induces trophoblast differentiation in mouse blastocyst outgrowths. *Cell Tissue Res.* 314:275.
34. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today* 20:561.
35. Smits, H. H., E. C. de Jong, J. H. Schuitemaker, T. B. Geijtenbeek, Y. van Kooyk, M. L. Kapsenberg, and E. A. Wierenga. 2002. Intercellular adhesion molecule-1/LFA-1 ligation favors human Th1 development. *J. Immunol.* 168:1710.
36. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2:151.
37. Dong, X., L. A. Bachman, R. Kumar, and M. D. Griffin. 2003. Generation of antigen-specific, interleukin-10-producing T-cells using dendritic cell stimulation and steroid hormone conditioning. *Transp. Immunol.* 11:323.
38. Joachim, R., A. C. Zenclussen, B. Polgar, A. J. Douglas, S. Fest, M. Knackstedt, B. F. Klapp, and P. C. Arck. 2003. The progesterone derivative dydrogesterone abrogates murine stress-triggered abortion by inducing a Th2 biased local immune response. *Steroids* 68:931.
39. Jiga, L. P., T. M. Bauer, J. J. Chuang, G. Opelz, and P. Terness. 2004. Generation of tolerogenic dendritic cells by treatment with mitomycin C: inhibition of allogeneic T-cell response is mediated by downregulation of ICAM-1, CD80, and CD86. *Transplantation* 77:1761.
40. Dubey, C., M. Croft, and S. L. Swain. 1995. Costimulatory requirements of naive CD4 $^{+}$  T cells. ICAM-1 or B7-1 can costimulate naive CD4 T cell activation but both are required for optimum response. *J. Immunol.* 155:45.
41. Luksch, C. R., O. Winqvist, M. E. Ozaki, L. Karlsson, M. R. Jackson, P. A. Peterson, and S. R. Webb. 1999. Intercellular adhesion molecule-1 inhibits interleukin 4 production by naive T cells. *Proc. Natl. Acad. Sci. USA* 96:3023.
42. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392:245.
43. Lutz, M., and G. Schuler. 2002. Immature, semi-immature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol.* 23:445.
44. Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and non-polarized T cells. *Nat. Immunol.* 1:311.
45. Suzuki, S., S. Tone, O. Takikawa, T. Kubo, I. Kohno, and Y. Minatogawa. 2001. Expression of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase in early concepti. *Biochem. J.* 355:425.
46. Grohmann, U., F. Fallarino, and P. Puccetti. 2003. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.* 24:242.
47. Jonuleit, H., E. Schmitt, G. Schuler, J. Knop, and A. H. Enk. 2000. Induction of interleukin 10-producing, nonproliferating CD4 $^{+}$  T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* 192:1213.
48. Lavelle, E. C., E. McNeela, M. E. Armstrong, O. Leavy, S. C. Higgins, and K. H. Mills. 2003. Cholera toxin promotes the induction of regulatory T cells specific for bystander antigens by modulating dendritic cell activation. *J. Immunol.* 171:2384.
49. Margni, R. A., and R. A. Binaghi. 1988. Nonprecipitating asymmetric antibodies. *Annu. Rev. Immunol.* 6:535.
50. Garcia-Lloret, M. L., B. Winkler-Lowen, and L. J. Guilbert. 2000. Monocytes adhering by LFA-1 to placental syncytiotrophoblasts induce local apoptosis via release of TNF- $\alpha$ : a model for hematogenous initiation of placental inflammations. *J. Leukocyte Biol.* 68:903.
51. Arias, R. A., L. D. Munoz, and M. A. Munoz-Fernandez. 2003. Transmission of HIV-1 infection between trophoblast placental cells and T-cells take place via an LFA-1-mediated cell to cell contact. *Virology* 307:266.
52. Xiao, J., M. Garcia-Lloret, B. Winkler-Lowen, R. Miller, K. Simpson, and L. J. Guilbert. 1997. ICAM-1-mediated adhesion of peripheral blood monocytes to the maternal surface of placental syncytiotrophoblasts: implications for placental villitis. *Am. J. Pathol.* 150:1845.
53. Dang, Y., J. Beckers, C. R. Wang, and K. Heyborne. 2000. Natural killer 1.1 $^{+}$   $\alpha\beta$  T cells in the periimplantation uterus. *Immunology* 101:484.
54. Sulila, P., R. Holmdahl, I. Hansson, F. Bernadotte, A. Mattsson, and R. Mattsson. 1988. An investigation of allogeneic pregnancy in nulliparous mice subjected to in vivo depletion of CD8 (Ly2)-positive lymphocytes by monoclonal antibody treatment. *J. Reprod. Immunol.* 14:235.
55. Basso, O., K. Christensen, and J. Olsen. 2001. Higher risk of pre-eclampsia after change of partner: an effect of longer interpregnancy intervals? *Epidemiology* 12:624.
56. Saftlas, A. F., R. J. Levine, M. A. Klebanoff, K. L. Martz, M. G. Ewell, C. D. Morris, and B. M. Sibai. 2003. Abortion, changed paternity, and risk of preeclampsia in nulliparous women. *Am. J. Epidemiol.* 157:1108.
57. Hedley, M. L., B. L. Drake, J. R. Head, P. W. Tucker, and J. Forman. 1989. Differential expression of the class I MHC genes in the embryo and placenta during midgestational development in the mouse. *J. Immunol.* 142:4046.
58. Redline, R. W., and C. Y. Lu. 1989. Localization of fetal major histocompatibility complex antigens and maternal leukocytes in murine placenta: implications for maternal-fetal immunological relationship. *Lab. Invest.* 61:27.
59. Isobe, M., H. Yagita, K. Okumura, and A. Ihara. 1992. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. *Science* 255:1125.
60. Moriyama, H., K. Yokono, K. Amano, M. Nagata, Y. Hasegawa, N. Okamoto, K. Tsukamoto, M. Miki, R. Yoneda, N. Yagi, et al. 1996. Induction of tolerance in murine autoimmune diabetes by transient blockade of leukocyte function-associated antigen-1/intercellular adhesion molecule-1 pathway. *J. Immunol.* 157:3737.
61. Takei, Y., Y. Nishimura, S. Kawano, H. Nagai, A. Ohmae, H. Fusamoto, and T. Kamada. 1996. Expression of ICAM-1 is involved in the mechanism of liver injury during liver transplantation: therapeutic usefulness of the F(ab') $_2$  fragment of an anti-ICAM-1 monoclonal antibody. *Transplant. Proc.* 28:1103.