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Interleukin 4 Production by Human Amnion Epithelial Cells and Regulation of Its Activity by Glycosaminoglycan Binding[1]

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ABSTRACT

The pro-inflammatory molecules, tumor necrosis factor α (TNFα), interleukin 1 (IL-1), interleukin 6 (IL-6), and prostaglandin E_2 (PGE_2), are postulated to have a role in human pregnancy and parturition. The ability of interleukin 4 (IL-4) to suppress the production of TNFα, IL-1, IL-6, and PGE_2 by activated monocytes prompted us to investigate a possible regulatory role for IL-4 in human gestation. Immunohistochemical techniques were used to show that human amnion epithelium stained positively for IL-4. Tissue from both the first (n = 5) and third (n = 46) trimester expressed immunoreactive IL-4, which was detected by the use of four antihuman IL-4 monoclonal antibodies. Analysis of mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR) on RNA extracts of amnion epithelial cells indicated that they were the source of IL-4. One of the anti-IL-4 antibodies used stained IL-4 protein associated with the basement membrane of the amnion epithelium. The mechanism of this association was investigated. IL-4 was shown to be a heparin-binding cytokine, which would enable it to bind to components of the extracellular matrix. Thus, this study identified a previously undescribed cellular source of IL-4, implicating a role for IL-4 in human gestation. Additionally, glycosaminoglycan binding may regulate IL-4 activity in vivo.

INTRODUCTION

Interleukin 4 (IL-4) is a key regulator of immune and inflammatory responses. Initially described as a T cell product [1], IL-4 is also produced by mast cells [2], bone marrow stromal cells [3], and basophils [4]. IL-4 was originally recognized as a B cell-stimulatory factor because it induced the proliferation of B lymphocytes in response to anti-IgM antibody [1]. The list of biological activities attributable to IL-4 continues to expand, and includes induction of the low-affinity IgE receptor (CD23) on B cells [5] and monocytes [6] and class II major histocompatibility complex molecules on B cells [7]. IL-4 induces the proliferation of a number of cell types, including fibroblasts [8] and T cells [9], and is recognized as a switch factor for the production of both IgE and IgG1 by B cells [10]. IL-4 has also been termed anti-inflammatory because of its ability to suppress tumor necrosis factor α (TNFα), IL-1, IL-6, and prostaglandin E_2 (PGE_2) production by activated monocytes [11].

Increases in bioactive TNF, IL-1, and IL-6 levels have been measured in the amniotic fluid of women in spontaneous labor at term compared to women at term, but not in labor [12]. Explants of postlabor decidual tissue have higher levels of TNFα in the culture supernatant than explants prepared from tissue collected before the onset of labor [13]. PGE_2 levels are also increased in the amniotic fluid in association with labor [14]. TNFα and IL-1 can stimulate PGE_2 production by human decidual explants [15] and human amnion epithelial cells [16], suggesting that there exists a network of mediators interacting to initiate and/or maintain human labor. Because decidual macrophages have been identified as one of the major sources of these mediators in utero and because IL-4 can suppress TNFα, IL-1, IL-6, and PGE_2 levels of activated monocytes [11], we postulated that IL-4 may similarly regulate the levels of these pro-inflammatory molecules at the materno-fetal interface.

Initially, IL-4 expression by the fetal membranes was examined through use of immunohistochemical staining with four anti-human IL-4 antibodies. Amnion epithelial cells stained positively with all four anti-IL-4 antibodies at all stages of gestation studied. Interestingly, we observed an association between IL-4 and the amniotic epithelium basement membrane. The potential for IL-4 to bind to components of extracellular matrix (ECM) was shown by heparin binding studies. Heparan sulphate and dermatan sulphate significantly inhibited IL-4-mediated suppression of lipopolysaccharide (LPS)-stimulated TNFα production by human monocytes. Thus, this report identifies a novel cellular source of IL-4, and is also the first account of the potential for IL-4 to bind to components of ECM. Furthermore, IL-4 activity is modulated by this binding.

MATERIALS AND METHODS

Tissue Samples

Third-trimester tissue (fetal membranes) was obtained after spontaneous vaginal deliveries or from elective cesarean sections. First-trimester tissue of 8–11-wk gestation, calculated by date of previous menstrual period, was obtained from elective pregnancy terminations. Tissue was collected

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and processed within 30 min of delivery. All tissue was collected with the approval of the Flinders Medical Centre Committee on Clinical Investigations.

Immunohistochemistry

Biopsies collected from the fetal membranes were rolled and frozen in liquid nitrogen-quenched isopentane. Specimens were stored at −70°C until sectioned. Pieces of tissue were embedded in O.C.T. compound (Tissue-Tek, Miles Labs., Elkhart, IN), and 8-mm sections were cut at the cryostat. Sections were air-dried overnight and then fixed in acetone for 10 min at room temperature. After incubation with 10% heat-inactivated normal swine serum (56°C, 30 min; CSL, Parkville, Australia), sections were incubated overnight with primary antibody. Four different anti-human IL-4 antibodies were used: 11B4.6 (culture supernatant, rat IgG1; kindly provided by Dr. M. Howard, DNAX, Palo Alto, CA), IL-4M1 (mouse IgG1, used at 12 μg/ml), IL-4M3 (mouse IgG2a, 10 μg/ml), and IL-4M8 (mouse IgG1, 8.5 μg/ml; generously provided by Dr. M.B. Widmer, Immunex, Seattle, WA). All antibodies had been characterized by a combination of immunoprecipitation, dot-blot, or neutralization by the suppliers. X63 (IgG1) and Sal 5 (IgG2a) (courtesy of Prof. H. Zola, Department of Immunology, Flinders Medical Centre, Australia) were used as isotype-matched controls for the murine antibodies. Diluted normal rat serum (1/10 000) was used as the negative control for 11B4.6. Anti-laminin (rabbit polyclonal antibody kindly provided by Dr. R. Rodgers, Department of Medicine, Flinders University of South Australia) and anti-collagen IV (rabbit polyclonal antibody generously donated by Dr. J. Stirling, Department of Histopathology, Flinders Medical Centre, Australia) antibodies were also used. After incubation with the appropriate biotin-conjugated goat anti-mouse or anti-rat immunoglobulin (Dakopatts, Glostrup, Denmark) or anti-rabbit immunoglobulin (Silenus Laboratories, Hawthorn, Australia) and peroxidase-conjugated streptavidin (Dakopatts), the reaction product was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St. Louis, MO). Sections were counterstained with hematoxylin solution, dehydrated, and mounted in DePeX (BDH, Kilsyth, Australia) for viewing.

Amnion Epithelial Cells

Single cell suspensions of amnion epithelial cells were prepared as described elsewhere [17]. Briefly, the amniotic membrane of placentas delivered by elective cesarean section was dissected from the fetal membranes. Amniotic tissue overlying the placenta was not used. Tissue was washed free of blood clots and cut into small pieces. Three digestions at 37°C for 30 min were performed in 0.25% trypsin (Difco, Detroit, MI) in Dulbecco's Minimum Eagle's medium (Sigma Chemical Co.) and Ham's F-12 (Cytosystems, Castle Hill, Australia), 1:1, supplemented with 2 mM MOPS (3-[*N*-morpholino]propanesulfonic acid; Sigma Chemical Co.), 2 mM glutamine, 250 mM $NaHCO_3$, 200 U/ml penicillin, and 200 μg/ml streptomycin (amnion epithelial cell culture medium). Supernatant from the first digest was discarded, and those from the second and third digests were pooled. Amnion epithelial cells were collected by centrifugation (600 × *g*, 10 min) of the pooled digests and were resuspended at 10^6 cells/ml in amnion epithelial cell culture medium containing 10% heat-inactivated fetal calf serum (FCS; Cytosystems). Cells were incubated in cell-nonadherent minisorp tubes (Nunc, Roskilde, Denmark) for 0, 2, or 24 h at 37°C in 5% CO_2; they were then resuspended at 5 × 10^5/ml and cytocentrifuge smears were prepared. After air-drying, the cytocentrifuge smears were fixed in acetone for 10 min; they were then stored at −20°C until used for immunohistochemistry. Amnion epithelial cells were routinely of > 98% purity by anti-CD59 (mouse monoclonal antibody generously provided by Dr. S. Parker, Department of Microbiology and Infectious Diseases, Flinders University of South Australia) flow cytometric analysis [18].

RNA Isolation

The amniotic membrane was dissected from the underlying layers of fetal membrane and uterine decidua. Total cellular RNA was extracted from homogenized tissue by the guanidine thiocyanate/phenol/chloroform procedure [19]. Duplicate samples were prepared.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR)

RNA isolated from 100 mg of tissue was reverse-transcribed as described elsewhere [20] and then stored at −20°C until analyzed by PCR. Primer sequences (5′ and 3′, respectively) and Mg^{2+} and deoxynucleotide triphosphate concentrations and cycle numbers were as follows: glyceraldehyde 3-phosphate dehydrogenase (GAPDH)— ACCACCATGGAGAAGACTGG, CTCAGTGTAGCCCAGGATGC, 1.5 mM, 200 μM, 25 cycles; IL-4— ATGGGTCTCACCTCCCAACTGCT, CGAACACTTTGAATATTTCTCTCTCAT, 0.5 mM, 100 μM, 35 cycles. Cycling parameters were 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. Each primer pair spanned at least one intron in the respective genomic sequence, allowing cDNA and genomic amplification products to be distinguished.

Southern Hybridization Analysis

After electrophoresis, PCR products were transferred to a nylon membrane (HYBOND-N+; Amersham, North Ryde, Australia) by Southern blotting [21]. The membranes were hybridized with an appropriate oligonucleotide internal to the PCR primers and were end-labeled with ^{32}P by standard techniques [21]. The sequences of the internal oligonucleotide probes were: GAPDH— GTGGAAGGACTCATGACCACAGTCCATGCC; IL-4— CTCAGTTGTGTTCTTGGAGGCA-

GCAAAGATG. After hybridization, membranes were exposed to a Storage Phosphor Screen and the screen was scanned on a Phosphor Imager (Series 400; Molecular Dynamics, Sunnyvale, CA).

Heparin Binding

Binding was assessed with a 1-ml heparin-Sepharose column (HI-TRAP; Pharmacia, Uppsala, Sweden). Recombinant human IL-4 (100 ng, 5×10^7 U/mg; Dr. S. Gillis, Immunex) was applied, allowed to bind for 30 min, and eluted with 0.2–1.0 M NaCl or 1–100 U/ml heparin (Delta West Limited, Bentley, Australia) in column buffer (PBS diluted 1:10 with water). One-milliliter fractions were collected and dialyzed against PBS; the IL-4 content was determined by the ability, during 24-h incubation, to increase CD23 expression and to suppress LPS-induced TNFα production by human monocytes. To assess the effect of glycosaminoglycan binding on IL-4 activity, serial dilutions of heparan sulphate (from bovine intestinal mucosa; Sigma Chemical Co.) and dermatan sulphate (chondroitin sulphate B, from bovine mucosa; Sigma Chemical Co.) were incubated with IL-4 for 30 min at room temperature and then placed in culture tubes with monocytes and LPS. Control cultures did not contain IL-4 but did contain heparan sulphate or dermatan sulphate.

Monocyte Isolation and Culture

Human peripheral blood monocytes were isolated under LPS-free conditions from buffy coats (Red Cross, Adelaide, Australia) by countercurrent centrifugal elutriation as previously described [11, 22]. Monocytes enriched to > 85% were resuspended in minisorp tubes (Nunc) at 10^6/ml in RPMI-1640 medium (Cytosystems) supplemented with 2 mM MOPS, 13.3 mM $NaHCO_3$, 2 mM glutamine, 50 mM 2-mercaptoethanol, 100 U/ml penicillin, and 100 mg/ml streptomycin. Fractions eluted from a heparin-Sepharose column were dialyzed and added to the monocyte cultures at a final concentration of 10% (v/v). LPS (500 ng/ml) was included in each culture; 10 ng/ml or 1 ng/ml recombinant human IL-4 (5×10^7 U/mg; Dr. S. Gillis, Immunex) was included as a positive control. After incubation for 24 h at 37°C in 5% CO_2, culture supernatants were removed for assay of TNFα levels. Cells remaining in the minisorp tubes were collected and stained for analysis of CD23 by flow cytometry.

Immunoassays

Immunoreactive TNFα was measured by a sandwich ELISA using a plate-binding monoclonal anti-TNFα antibody and a second rabbit polyclonal anti-TNFα antibody (kindly provided by Prof. A.C. Allison, Syntex, Palo Alto, CA) as described elsewhere [11, 23]. The assay was sensitive to levels of TNFα greater than 0.04 ng/ml. Immunoreactive IL-4 was measured by a sandwich ELISA using a matched pair of antibodies (Pharmingen, San Diego, CA) according to the manufacturer's instructions. The assay was sensitive to IL-4 levels > 0.03 ng/ml.

CD23 Expression

Cell suspensions from triplicate culture tubes were pooled and centrifuged. Pelleted cells were resuspended in 50 μl normal human immunoglobulin (5 mg/ml; CSL). After incubation for 30 min at 4°C, 5 μl of anti-CD23 monoclonal antibody (B6; Coulter Immunology, Hialeah, FL) was added for a further 30 min. After being washed in PBS/0.02% sodium azide, cells were resuspended in 50 ml fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (1:40, Sigma Chemical Co.) for 30 min at 4°C. Cells were then washed and resuspended in 100 ml PBS supplemented with 1% formaldehyde, 2% glucose, and 5 mM sodium azide. Cell suspensions were stored at 4°C until flow cytometric analysis, which was conducted within 1 wk (FACScan; Becton, Dickinson and Co., Mountain View, CA).

Expression of Results

TNFα measurements were performed on samples from triplicate cultures, and the mean values from each set of triplicates were used to determine the mean ± SEM for n donors. For flow cytometric analysis of CD23 expression, means were calculated from single cell suspensions for n donors.

Statistical Analysis

The significance of difference between treatment groups was assessed through use of a two-tailed Student's *t*-test. Results were considered significantly different if $p < 0.05$.

RESULTS

IL-4 Detection in Amnion Epithelium

Amnion epithelial cells stained positively for IL-4 with three different mouse anti-human IL-4 monoclonal antibodies. The amnion epithelium stained positively with each antibody in 46 of 46 third-trimester samples; these samples ranged from 28 wk of gestation to term. IL-4 staining was observed in term samples obtained before the onset of labor (elective cesarean section) and in samples obtained after the completion of labor (spontaneous vaginal delivery). First-trimester tissue samples (8–11 wk of gestation) also stained positively for IL-4 (n = 5). Figure 1 shows sections from a term elective cesarean section stained with (A) X63 (mouse IgG1 control) and (B) M8 (mouse IgG1 anti-human IL-4). M1 and M3 antibodies gave the same pattern of immunoreactivity to amnion epithelial cells as did M8 (Fig. 1B). A fourth anti-human IL-4 antibody (11B4.6), although positively identifying IL-4 protein in amnion epithelium, gave a different pattern of staining. Figure 1C shows the negative

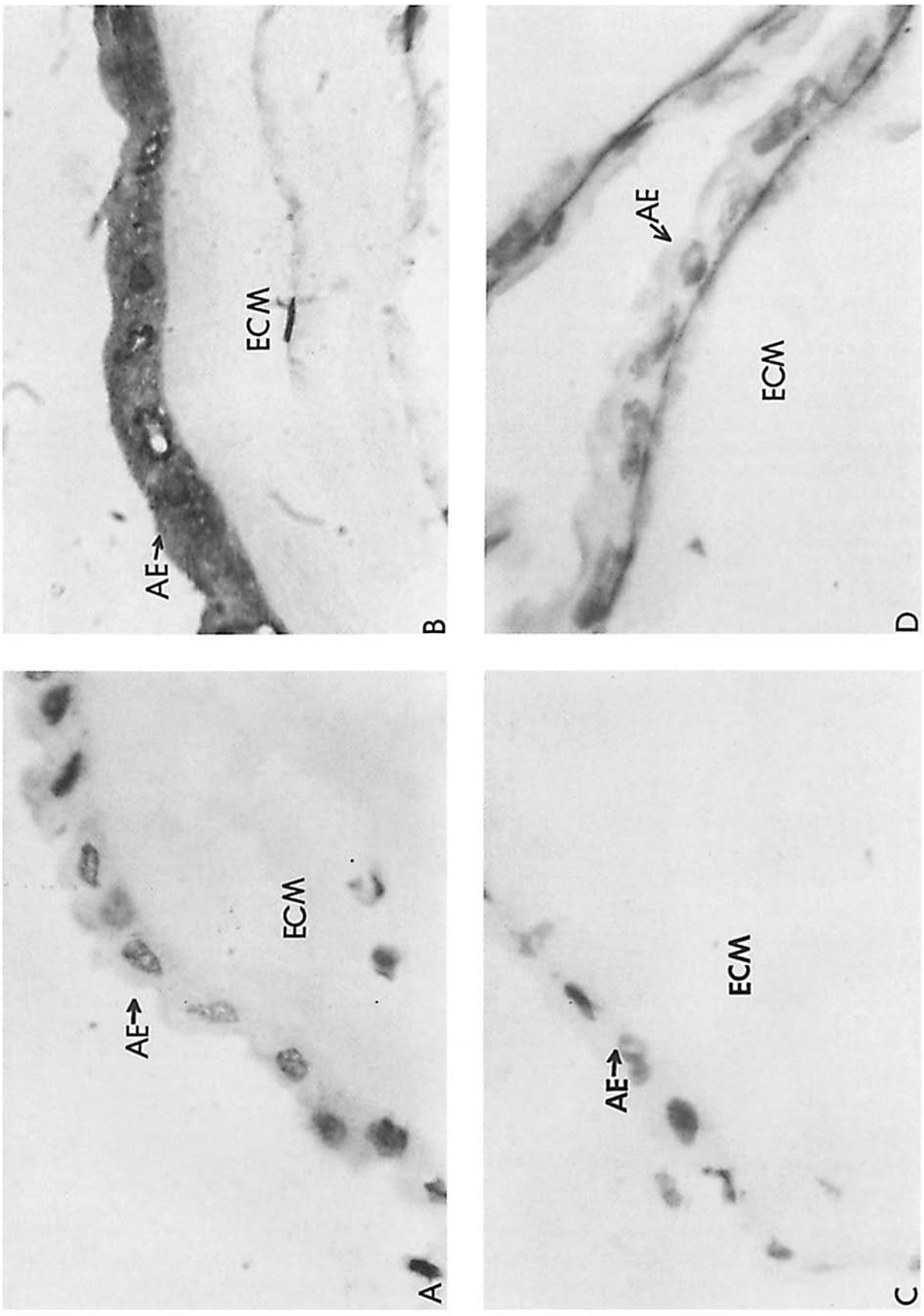

FIG. 1. Amnion epithelium expression of immunoreactive IL-4 protein. Eight-micrometer sections of fetal membranes were stained with (A) X63, the isotype matched control for M8, (B) M8 (mouse anti-human IL-4; IgG1), (C) diluted rat serum as a negative control for 11B4.6, and (D) 11B4.6 (rat anti-human IL-4; IgG1). AE, amnion epithelium, ECM, extracellular matrix. Magnification ×5000. (Original magnification ×7600.)

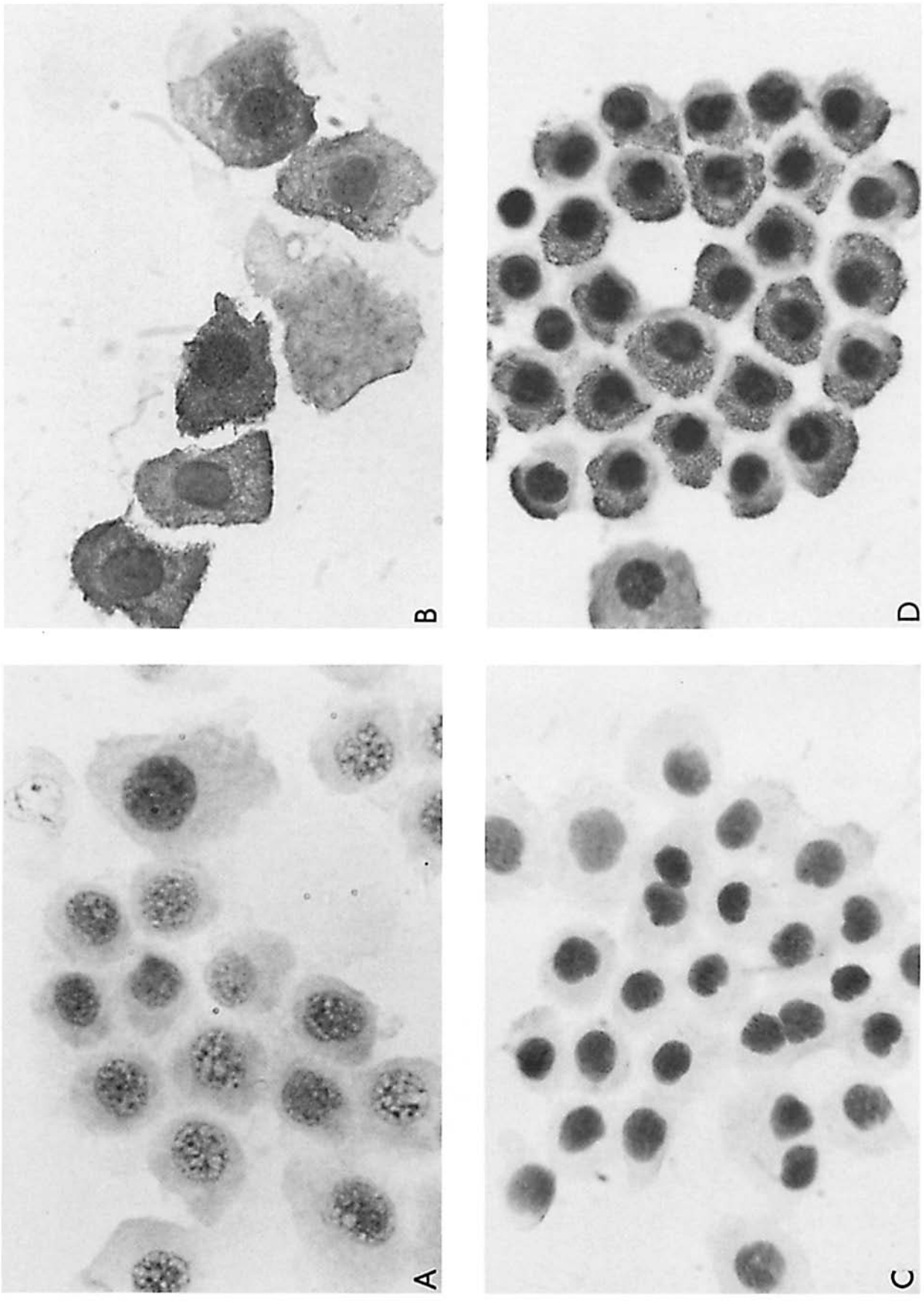

FIG. 2. Amnion epithelial cell expression of immunoreactive IL-4 protein. Single cell suspensions were prepared by trypsinization of amniotic membrane as described in *Materials and Methods*. Cytocentrifuge smears of these preparations were stained at 0, 2, and 24 h with (A) *X63, the isotype-matched control for M8, (B) M8 (mouse anti-human IL-4; IgG1), (C) diluted rat* serum as a negative control for 11B4.6, and (D) 11B4.6 (rat anti-human IL-4; IgG1). Magnification ×5000. (Original magnification ×7600.)

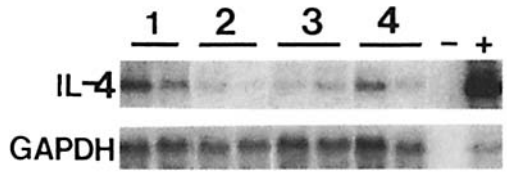

FIG. 3. Amnion epithelium expression of IL-4 mRNA as determined by Southern hybridization of PCR products. Total cellular RNA was extracted from homogenized amnion epithelium (n = 4) and IL-4 mRNA was determined by RT-PCR and Southern hybridization. All samples were prepared in duplicate. Human lymphocytes stimulated for 24 h with phorbol 12-myristate 13-acetate (10 ng/ml) and phytohemagglutinin (1 μg/ml) were used as a positive control (+). The negative control (−) was the RT-PCR product of a water sample. GAPDH mRNA was analyzed to evaluate consistency between preparations.

control in which diluted rat serum was used, and Figure 1D shows tissue from the same sample stained with 11B4.6. Cytoplasmic staining was weak, but strong staining was observed in association with the basement membrane of the amnion epithelium. Immunostaining with antibodies to laminin and collagen IV confirmed that the site of reactivity for 11B4.6 was the basement membrane (data not shown).

Cytosmears prepared from single cell suspensions of human amnion epithelial cells cultured for 0, 2, or 24 h were stained with the M8 antibody or 11B4.6. At 0, 2, and 24 h, all preparations (n = 7) showed intracellular staining with the M8 antibody (Fig. 2B). Figure 2A shows the isotype-matched control (X63). With use of the 11B4.6 anti-human IL-4 antibody, 6 of 7 samples stained positively at 0 h (Fig. 2D). It is uncertain whether the staining was intracellular or whether it reflected surface staining on a multidimensional cell. Figure 2C shows the negative control.

Immunoreactive IL-4 was detected in lysates of cell suspensions of amnion epithelial cells (292 ± 60 pg/10^6 cells, mean ± SD, n = 4).

Total RNA was extracted from the amnion epithelium that can be readily separated from the underlying choriodecidua. RT-PCR and Southern hybridization with a ^{32}P-labeled oligonucleotide internal to the PCR primers were used to assess the presence of IL-4 mRNA. Figure 3 shows that IL-4 mRNA was detectable from all preparations of amnion epithelial cells (n = 4).

The Heparin-Binding Ability of IL-4

After incubation on a heparin-Sepharose column for 30 min, IL-4 was eluted with increasing concentrations of NaCl. The effect of eluted fractions on 1) CD23 expression and 2) LPS-stimulated TNFα levels of human monocytes was examined (n = 3). Figure 4A shows that fraction 3, which eluted at 0.5 M NaCl, increased CD23 expression of human monocytes. There was a concomitant suppression of LPS-induced TNFα levels of human monocytes treated with fraction 3 (Fig. 4B), further suggesting that IL-4 was eluted from heparin-Sepharose by 0.5 M NaCl. IL-4 was also eluted from heparin-Sepharose by 100 U/ml heparin (Fig. 5). IL-4 did not bind nonspecifically to Sepharose-CL-6B (data not shown).

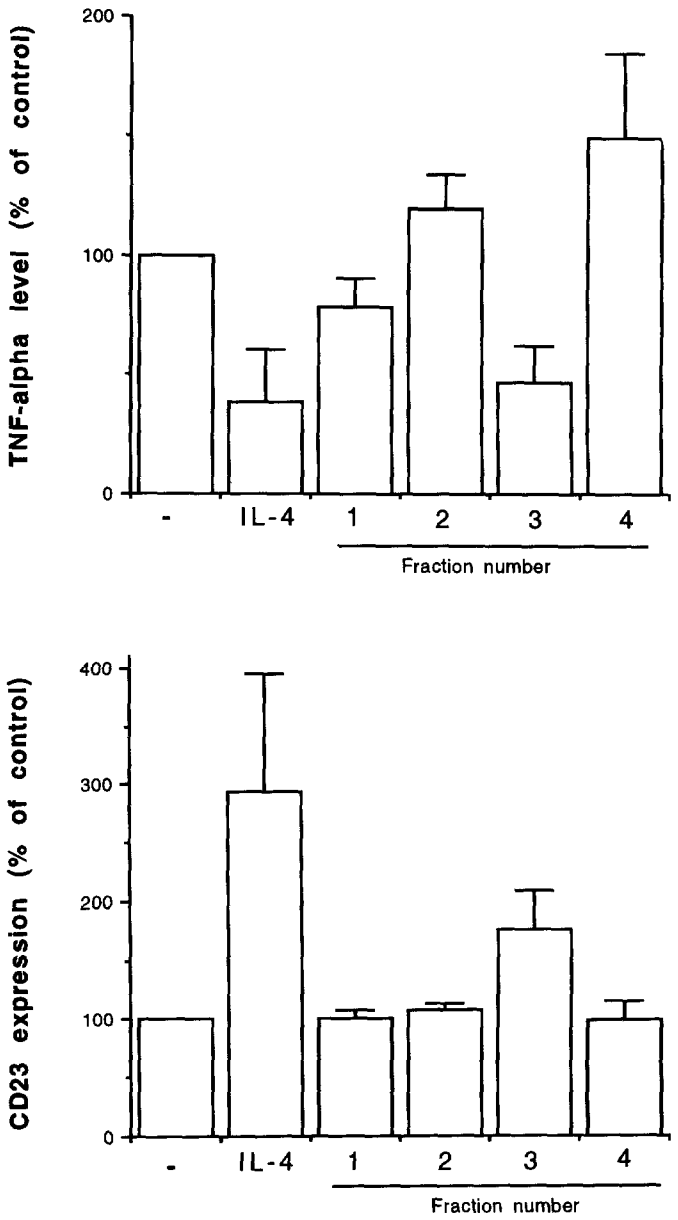

FIG. 4. The heparin-binding ability of IL-4. After 30-min incubation of IL-4 with a heparin-Sepharose column, fractions were eluted with increasing concentrations of NaCl (0.2–1.0 M). In three different experiments, IL-4 activity was assessed by evaluation of (A) TNFα levels (mean ± SEM) and (B) CD23 expression (mean ± SEM) of LPS-stimulated (500 ng/ml) monocytes. Fractions: 1, eluted with column buffer; 2, with 0.2 M NaCl; 3, with 0.5 M NaCl; and 4, with 1.0 M NaCl.

The Effect of Glycosaminoglycans on IL-4 Activity

Figure 6 shows the effects of incubating IL-4 with dermatan sulphate or heparan sulphate for 30 min prior to incubation of IL-4 with human peripheral blood monocytes. The final concentration of IL-4 in each culture was 1 ng/ml. Cultures were terminated after 24 h; supernatants

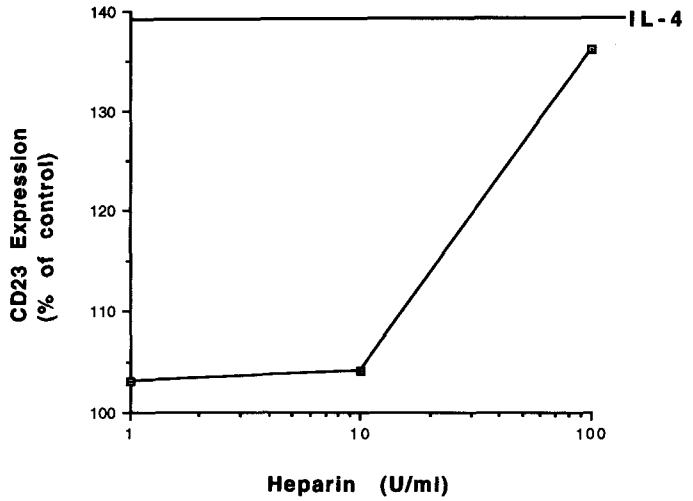

FIG. 5. The effect of heparin on IL-4 binding to heparin-Sepharose. IL-4 was eluted from heparin-Sepharose with increasing concentrations of heparin (1–100 U/ml). IL-4 activity was assessed by the induction of CD23 expression by human monocytes. IL-4 was included as a positive control for CD23 expression. A representative experiment (of three) is shown.

were assayed for TNFα and cells analyzed for CD23 expression. Typically, dermatan sulphate and heparan sulphate alone had negligible effect on CD23 expression and LPS-induced TNFα levels of human monocytes. However, results are presented as levels of CD23 and TNFα (in cultures containing IL-4) as a percentage of the levels in cultures not containing IL-4, matched for glycosaminoglycan content. Figure 6A shows that through pre-incubation of IL-4 (1 ng/ml) with 10 μg/ml (n = 7) or 1 μg/ml (n = 6) of heparan sulphate, IL-4-mediated suppression of LPS-induced TNFα was significantly inhibited ($p = 0.04$ and $p = 0.03$, respectively). Dermatan sulphate doses of 10 μg/ml (n = 5) and 1 μg/ml (n = 5) also significantly reduced IL-4 inhibition of LPS-induced TNFα levels ($p = 0.04$ and $p = 0.03$, respectively). In contrast, both heparan sulphate and dermatan sulphate were without any effect on the induction of CD23 expression by human monocytes in response to IL-4 (Fig. 6B).

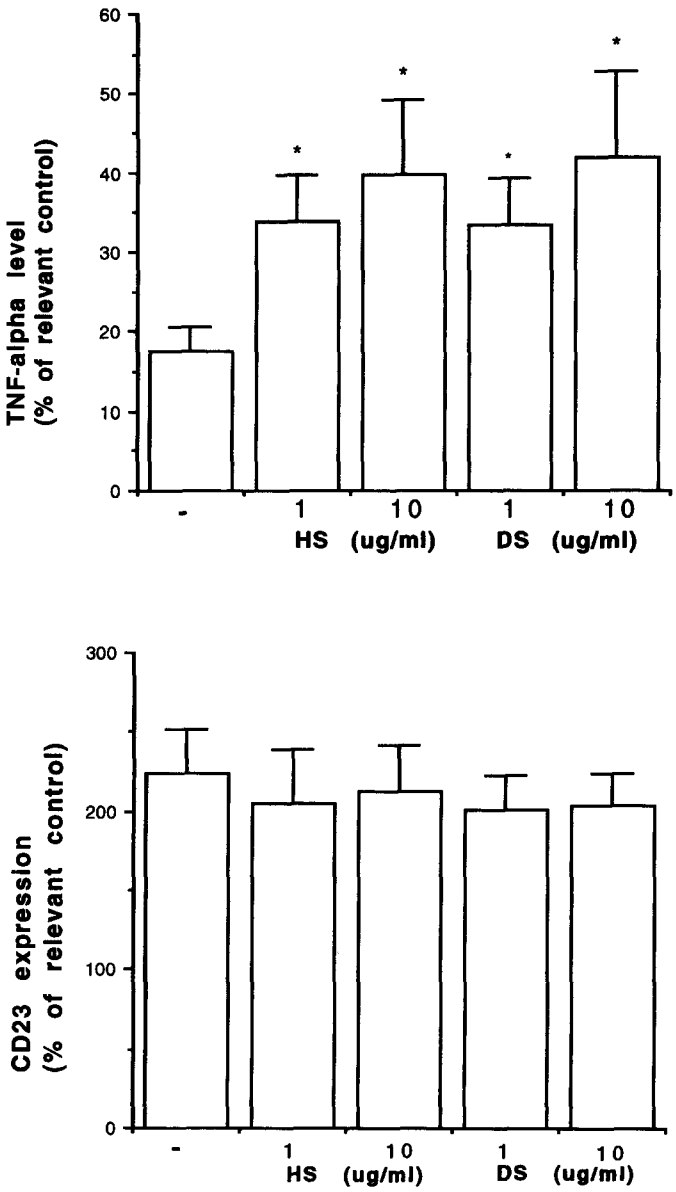

FIG. 6. The effect of glycosaminoglycan binding on IL-4 activity. IL-4 preincubated for 30 min with heparan sulphate (HS) or dermatan sulphate (DS) was incubated with human monocytes and LPS (500 ng/ml) for 24 h. Changes in IL-4 activity were assessed by measuring (A) IL-4-induced suppression of LPS-stimulated TNFα levels and (B) IL-4-induced expression of CD23. Note that results (mean ± SEM, n = 5, 6, or 7) are presented relative to expression in cultures containing an equal amount of HS or DS, but no IL-4. An asterisk denotes a significant difference from the control value ($p < 0.05$).

DISCUSSION

In the present study, a previously unrecognized cellular source of IL-4, the amnion epithelium, was identified. Amnion epithelial cells expressed both IL-4 protein (Fig. 1B, 2B, and 2D) and IL-4 mRNA (Fig. 3). Amnion epithelial cells were found to be IL-4-positive throughout the third trimester of human gestation. IL-4 was expressed in samples obtained both before and after labor; however, further study is required to assess quantitative differences between prelabor and postlabor samples. First-trimester tissue of 8–11-wk gestation also stained positively for IL-4, suggesting that IL-4 is expressed throughout pregnancy from at least the eighth week of gestation on.

Three of the anti-human IL-4 antibodies used in this study identified cytoplasmic IL-4 in amnion epithelial cells. A fourth antibody (11B4.6) gave a different pattern of staining: strong staining in association with the cell periphery (Fig. 1D and 2D) with weak cytoplasmic staining on cryostat-prepared sections of tissue. These results indicate that IL-4 from amnion epithelial cells may remain in intimate contact with its cell of origin, suggesting an autocrine or juxtacrine/paracrine role for this cytokine. We hypothesize that the ap-

parent polarity arises as a consequence of binding of IL-4 to a component of the basement membrane that allows localization of IL-4 at this site. A similar anomaly in staining patterns with anti-human IL-4 antibodies was observed by Bradding et al. [24] in a study of human nasal mucosa. One of the antibodies used in that study (3H4) gave weak cytoplasmic staining but strong peripheral ring staining of mast cells. The authors postulated that this pattern of staining resulted from 1) intracellular IL-4 located at the cell periphery, 2) plasma membrane-bound IL-4, or 3) extracellular IL-4 maintained in close proximity to the cell surface via complexing with ECM components. The pattern of staining we have observed with 11B4.6 (Fig. 1D) is highly suggestive of an association of IL-4 with the amnion epithelial cells via a similar mechanism. Figure 2D raises the possibility that IL-4 is associated with the amnion epithelial cell membrane rather than the basement membrane. However, the similarity of the staining pattern obtained with 11B4.6 to that obtained with antibodies to two well-characterized components of the basement membrane, laminin and collagen IV [25], suggests that the staining observed on dispersed cells with 11B4.6 may result from remnants of basement membrane attached to the cell. Ongoing work is being conducted in an attempt to clarify this.

The production of IL-4 by amnion epithelial cells suggests a role for IL-4 in human gestation. Our investigations were prompted by the ability of IL-4 to suppress TNFα, IL-1, IL-6, and PGE_2 production by activated monocytes [11]. Increases in these pro-inflammatory molecules have been observed in amniotic fluid and gestation-associated tissues from women in labor compared to those not in labor [12, 13]. Marked elevation of these pro-inflammatory mediators is also observed in preterm labor (< 37 wk gestation) associated with genital tract infection [26]. Thus, a role for TNFα, IL-1, IL-6, and PGE_2 in the onset and/or maintenance of human labor has been postulated [12, 26], although causality has not been determined. We hypothesized that IL-4 may function as a regulator of the production of these pro-inflammatory molecules to prevent untimely labor and delivery. For example, as TNFα and IL-1 can induce PGE_2 production by amnion epithelial cells [16], one of the biological effects of IL-4 in utero may be to maintain pregnancy by preventing untimely production of PGE_2 by the amniotic epithelium. However, because we have observed that IL-4 is present in association with the amniotic epithelium after labor, IL-4 alone is unlikely to prevent the elevation of PGE_2 associated with labor. Future work in this laboratory will explore the roles for IL-4 at the materno-fetal interface.

On the basis of murine studies, in which IFNγ and IL-2—characterisitic T helper (Th) 1 cytokines—were found to compromise the progression of pregnancy [27], Wegmann et al. [28] have also recently postulated that Th 2-type cytokines may, in contrast, have a role in the maintenance of mammalian pregnancy. In support of this hypothesis, IL-4 and IL-10 were found to be produced by gestation-associated tissue collected from mice with normal pregnancies [29]. Similarly, our observation of IL-4 expression by human amnion epithelial cells from at least 8 wk of gestation supports a role for "Th2" cytokines as pregnancy-enhancing. Furthermore, our studies also suggest that we must not limit the sources of "Th2" cytokines to T lymphocytes [29].

The possibility that IL-4 binds to the basement membrane, via interactions with ECM components, was explored through assessment of the heparin-binding ability of IL-4 using a heparin-Sepharose column. Numerous other cytokines bind ECM components, including transforming growth factor ß (TGFβ) [30], fibroblast growth factor (FGF) [31], granulocyte macrophage/colony-stimulating factor (GM-CSF) [32], IL-8 [33], IL-3 [34], and TNFα [35]. In the present study, IL-4 was found to bind to heparin-Sepharose, from which it could be eluted with 0.5 M NaCl (Fig. 4). This is comparable to the heparin-Sepharose-binding activity observed for IL-8 [33] and TNFα [35].

ECM binding by cytokines has been suggested to have a number of consequences. Lantz et al. [35] found that TNFα could be dissociated from heparin-Sepharose by TNFα-binding protein. It was postulated that, in vivo, receptor-bearing cells would cause dissociation of cytokines from ECM. Thus, ECM binding may serve to localize cytokine activity in vivo. The association of IL-4 with the basement membrane could provide a mechanism for the immobilization of IL-4 at the amnion epithelium, suggesting juxtacrine activity. Additionally, ECM binding may modulate the half-life of cytokines. ECM binding has been shown to protect FGF from proteolytic degradation [36]. Similarly, a neutralizing anti-IL-4 antibody complexed with mouse IL-4 enhanced IL-4 activity in vivo, presumably by increasing the half-life of the cytokine by protecting the active site from proteolytic degradation [37]. Another consequence of cytokine binding to ECM is alteration of activity. For example, TGFβ activity is abrogated by binding with the proteoglycan, decorin [30]. We investigated the ability of two of the commonly occurring glycosaminoglycans, heparan sulphate and dermatan sulphate, to affect IL-4 activity. Interestingly, we found that both glycosaminoglycans inhibited IL-4-mediated inhibition of TNFα immunoreactivity from LPS-stimulated monocytes but were without effect on IL-4-induced CD23 expression by the same population of cells (Fig. 6).

Cytokines are postulated to bind to either the protein core or the glycosaminoglycan side chains of proteoglycans. Regions rich in positively charged amino acids, such as lysine, are postulated to contribute to ECM binding [37]. For example, the heparin-binding site for IL-8 has been demonstrated to reside in the lysine-rich C-terminal α-helix. However, the interaction between a cytokine and the ECM is thought to be more complex than ionic binding, as cytokines bind to different combinations of ECM components [38]. For example, IL-8 activity is altered by heparan sulphate but not chondrotin sulphate [31]. Recently, Wlo-

dawar et al. [39] identified patches of positively charged residues on the surfaces of helices C and D of IL-4. These were postulated to be potential receptor-binding sites. However, they can also serve as candidates for the heparin-binding site. The ability of IL-4 to bind other glycosaminoglycans, namely hyaluronic acid, chondroitin sulphate (A and B), and keratan sulphate, will be explored.

In summary, we have not only identified a previously unrecognized cellular source of IL-4 (human amnion epithelial cells), but also identified a novel mechanism for regulation of IL-4 bioactivity. The presence of IL-4 in association with the amnion epithelium implies a role for IL-4 in human gestation. However, when we consider the functions of IL-4 at this tissue site, we must do so in the context of ECM binding, which may serve to regulate IL-4 activity at the materno-fetal interface.

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