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ScienceDirect
Molecular Immunology 44 (2007) 1737–1742
Molecular Immunology
www.elsevier.com/locate/molimm

Synthesis of complement proteins in the human chorion is differentially regulated by cytokines

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Received 22 June 2006; received in revised form 13 July 2006; accepted 24 July 2006
Available online 26 September 2006

Abstract

The aim of the current paper was to determine the chorion's contribution to complement synthesis in the placenta and its regulation by cytokines. Biosynthetic labeling followed by immunoprecipitation with polyclonal antibodies was performed in chorionic tissue and chorion-derived cells. Eight complement proteins, factor B, C3, C1r, C1s, C1 inhibitor, factor H, C4 and C2 were detected in chorionic tissue and were secreted extracellularly. In chorion-derived cells, IL-1β stimulated factor B synthesis but had no effect on C1r, C1 inhibitor, C1s, factor H and C4. TNFα had no stimulative effect on any of the complement proteins tested. In contrast, both IL-1β and TNFα highly induced IL-6 secretion in chorion-derived cells, demonstrating the overall responsiveness of these cells to these stimuli. Interestingly, IFN-γ increased the synthesis of C1s, C1r, C1 inhibitor, C4 and factor H in chorion-derived cells. The fact that the latter two complement proteins have opposing effects on immune activation of the complement cascade demonstrates the complex balance required to both maintain an ability to ward off infections but simultaneously suppress the immune response to enable tolerance of the allograft fetus.

Keywords: Chorion; Complement; Cytokines

1. Introduction

The chorion is made up of two parts; the chorionic laeve cells derived from the mesoderm and the trophoblast cells derived from the ectoderm. The trophoblasts are bathed in maternal blood and form the reactive interface between the mother and her semiallogeneic fetus. Thus, the placenta requires strategies to protect itself against attack by the cellular and humoral elements of the maternal immune system.

In order to evade the damaging attack by the maternal immune response, trophoblasts develop various strategies including expression of non-classical MHC class I antigens and of complement regulatory proteins CD46, CD55, and CD59 (Bulla et al., 2003). Complement is an early effector in the pathway leading to pregnancy loss associated with placental inflammation (Girardi and Salmon, 2003). Furthermore, it appears that inhibition of complement activation is a requirement for normal pregnancy. For example, in the antiphosphospholid syndrome overwhelming activation of complement C3 triggered by antibodies deposited in the placenta leads to fetal injury (Holers et al., 2002). Similarly, in a mouse model, deficiency of a murine complement regulator, Crry, resulted in decreased survival for their embryos, in a C3 dependent manner (Xu et al., 2000).

The ability to regulate complement activation must be finely tuned to the hosts ability to mount an immune response against invading pathogens. Activation of complement is integral to the hosts natural response to infection by inducing chemotaxis, enhancing phagocytosis by neutrophils, facilitating immune complex clearance, and mediating cell lysis by the membrane attack complex. Many of the complement proteins can be detected in amniotic fluid. In healthy term newborns reference values in umbilical cord blood for C1r, C2, C5, C7, properdin, factor D, factor H, and factor I were measured (Sonntag et al., 1998). In addition, reference values for the concentration of activated split products C3A and C5A were devised (Sonntag et al., 1998).

The extreme cellular complexity of the placenta, however, has made it difficult to determine which complement proteins are produced by which cells. We approached this issue by measuring complement levels in independently isolated amnion and

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doi:10.1016/j.molimm.2006.07.298

chorion tissue and from cells derived therefrom. In previous studies, we reported that at term pregnancy, amnion tissue and cells synthesize and secrete factor H, C1r, C1s, C1 inhibitor, C3 and factor B (FB) (Katz et al., 1995). The latter, interestingly, is not expressed in fetal tissue in early gestation (Hasty et al., 1993). In this study, we investigated the contributions of the chorion to the synthesis and secretion of complement proteins in the term placenta. Furthermore, the capacity of the chorion to respond to the inflammatory cytokines IFN-γ, IL-1, and TNF-α was determined.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), Hanks' balanced salt solution (HBSS), trypsin, penicillin–streptomycin, L-glutamine and heat-inactivated fetal calf serum (FCS) were purchased from Biological Industries (Bet-Haemek, Israel). L-[^{35}S]methionine (11.0 mCi/ml) was purchased from NEN (Boston, MA). DMEM without methionine, cysteine L-glutamine, bovine serum albumin (BSA), collagenase (Type IA), leupeptin, PMSF, Triton X-100, sodium deoxycholate, TEMED, trichloroacetic acid and nonradioactive molecular weight markers were acquired from Sigma Chemical Co. (St. Louis, MO). Goat polyclonal antibodies to human C1r, C1s, C1 inhibitor (C1i), C3, C4, C2, factor B (FB) and factor H were obtained from DiaSorin Inc. (Stillwater, MN). Recombinant human IL-1β, recombinant human tumor necrosis factor-α (TNF-α), recombinant human IFN-γ and goat polyclonal antibodies to human IL-1β and IL-6 were purchased from R&D Systems (Minneapolis, MN). Protein A Pansorbin cells was purchased from Calbiochem Novabiochem (La Jolla, CA). Autofluor was purchased from National Diagnostics (Manville, NJ) and Ultima Gold from Packard (Downers Grove, IL). Sodium dodecyl sulfate (SDS), polyacrylamide, bis-acrylamide, ammonium persulfate, Tris and glycine were purchased from USB (Cleveland, OH).

2.2. Tissue collection and stimulation

The protocol was approved by the Helsinki committee of the institution and tissue was obtained after consent. Thirteen placentas were processed for these experiments. Amnion and chorion membranes were mechanically separated within 30 min after a normal full term uncomplicated vaginal delivery ($n=6$) or after elective term caesarian sections ($n=7$), as previously described (Katz et al., 1995). The separation was such that the amnion layer was undamaged and completely separated from the chorion layer. The respective tissues were thoroughly washed five times in phosphate buffered saline to remove blood clots and blood cells, and placed in culture within 90 min of delivery.About 350 mg pieces of the chorion or amnion avascular tissue explant were incubated in a given well of a 24-well cell culture (Costar, Corning, NY) cluster for 4 h and overnight in DMEM with 0.1% BSA alone (control, C), or containing IL-1β (1 ng/ml), TNF-α (5 ng/ml), or IFN-γ (5000 U/ml). Three wells were used for each condition.

2.3. Isolation of chorion-derived cells

Monolayers of chorion-derived cells were prepared by finely mincing the tissue and digesting with collagenase in DMEM (2.5 mg/ml) at 37 °C for 3 h, followed by addition of trypsin (0.25%) for additional 30 min. After neutralization of trypsin digests with 10% FCS in DMEM and centrifugation, the cells were washed, resuspended in DMEM with 10% FCS at approximately 300,000 cells/ml and plated in 24-well culture plates. Cultures were incubated at 37 °C in 5% CO_2 and after 24 h the media were replaced with fresh media. At confluence, usually after 4–5 days, the cells were prepared for the stimulation experiments under identical conditions as the tissue explants. The cells were obtained from nine different placenta preparations.

2.4. Biosynthetic labeling and immunoprecipitation

Biosynthetic labeling was performed essentially as previously described (Katz et al., 1995). In brief, after incubation of the tissue for 4 h or overnight, the explants were washed in HBSS and incubated in DMEM without methionine supplemented with [^{35}S]methionine (350 μCi/ml) for 150 min. IL-1β (1 ng/ml), TNF-α (5 ng/ml), IFN-γ (5000 U/ml) or carrier alone was present during the labeling period, as indicated. The chorion-derived cells were biosynthetically labeled under the same conditions. After completion of the pulse, the supernatants from the three wells of a given condition were pooled and the tissue or the cells were washed in PBS. Lysates of the tissue or the cells were prepared by freeze thawing (three times for the tissue) in the presence of Triton X-100 (0.5%), deoxycholate (0.25%), PMSF (2 mM), leupeptin (0.1 mg/ml) and EDTA (10 mM) followed by centrifugation at 100,000 × g for 15 min. Three wells of a given condition were pooled and the total protein synthesis was determined from the incorporation of [^{35}S]methionine into trichloroacetic acid insoluble protein. Proteins under study were sequentially immunoprecipitated from the tissue lysates, cell lysates and from the extracellular media with 3 μg of their respective polyclonal antibodies and protein A and then subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography. Incorporation of [^{35}S]methionine into the individual immunoprecipitated proteins was determined by liquid scintillation counting of gel slices after digestion with 15% hydrogen peroxide for 16 h at 65 °C and addition of Ultima Gold. The background was then subtracted to obtain the specific protein counts for each complement protein. To correct for the total amount of protein synthesized in each sample, the specific counts were divided by the total trichloroacetic acid (TCA) immunoprecipitable counts for that sample.

2.5. Statistical analysis

The significance of differences between groups under consideration was determined by two-tailed Student's t-test.