L-selectin and E-selectin expressed on monocytes mediating Ehrlichia chaffeensis attachment onto host cells

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Abstract

Ehrlichia chaffeensis, the agent of human monocytic ehrlichiosis, is an obligatory intracellular bacterium that exhibits monocytic host cell tropism. Ehrlichiae must enter the host cell, and then establish infection. The tropism of E. chaffeensis for monocytes suggests that the cell contains some specific surface components that mediate E. chaffeensis attachment and entry into host cells. In this study, host cell surface components that play a role in ehrlichial attachment were identified using a human monocyte/macrophage cell line, THP-1. E. chaffeensis attachment to THP-1 cells was partially blocked in the presence of antibodies to E-selectin and L-selectin, but not by antibodies to P-selectin, integrin αm, integrin αx, or normal mouse IgG as determined by real time polymerase chain reaction. Conversely, in HeLa cells that do not exhibit surface expression of E-selectin and L-selectin, antibodies to these cell surface proteins did not inhibit E. chaffeensis attachment. These findings indicate that E-selectin and L-selectin are cell surface proteins that might act as co-receptors and contribute to E. chaffeensis attachment and entry into THP-1.

Keywords: Ehrlichia chaffeensis; Selectin; Receptor

1. Introduction

Human monocytic ehrlichiosis (HME) is a newly emerging tick-borne infectious disease caused by Ehrlichia chaffeensis [1,2]. HME is a moderate to severe illness, life-threatening in some cases with a case fatality rate of 3% [3–8]. E. chaffeensis is an obligate intracellular bacterium, which resides in an early-endosomal vacuole primarily in the monocytes and macrophages [9]. E. chaffeensis enters the monocytes/macrophages by attachment to as yet unknown surface protein(s), which might serve as receptors for entry. Three types of bacterial adhesin–receptor interactions have been described for bacterial attachment to host cells [10]. The most commonly described are lectin–carbohydrate interactions, whereby bacterial surface polysaccharides or lipopolysaccharides bind to cognate lectins on the host cell surface. The second consists of an adhesin–receptor interaction that involves recognition of a protein on the bacteria by a complementary protein on the host cell surface. Binding interactions between hydrophobic moieties of proteins on one cell with lipid ions on the other cell or between the lipids on both cells have also been described.

Monocyte surface proteins with potential to serve as receptors for bacterial attachment include CR1, CR3, integrin (p150), Fc receptor, lectins and oligosaccharides. CR1, CR3 and p150 are complement receptors. Fc receptor is involved in phagocytosis of antibody-opsonized bacteria, which possibly has no role in the primary infection. Lectins are the receptors for oligosaccharides, and may be the receptors for E. chaffeensis since E. chaffeensis contains surface glycoproteins [11]. Selectins include E-selectin (CD62E), L-selectin (CD62L), and P-selectin (CD62P), which are cell adhesion molecules critically involved in the regulation of leukocyte traffic that bind a carbohydrate moiety mediating adhesion of leukocytes to endothelial cells. Each of the selectin molecules is a single chain transmembrane glycoprotein with a similar modular structure, including an extracellular calcium-dependent lectin domain on the amino-terminus, followed by a epidermal

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growth factor-like domain, a SCR domain consisting of two to nine short consensus repeats, a membrane spanning region, and a cytoplasmic tail [12].

Integrins are a major group of adhesion molecules, present on many cells, including leukocytes that have been identified as receptors for other bacteria [13] and could potentially serve as the receptor for \( E. \) chafeensis. Integrins are heterodimeric proteins composed of two non-covalently linked polypeptide chains, \( \alpha \) and \( \beta \). Integrins are classified into seven subfamilies, depending on their \( \beta \) chains. Of particular interest is the \( \beta 2 \) subfamily, which includes CD11aCD18 (FLA-1), CD11bCD18 (Mac-1, CR3), and CD11cCD18 (p150, p95, CR4), CD11 referring to different \( \alpha \) chains and CD18 to the common \( \beta 2 \) subunit. \( \beta 2 \) integrins are involved in leukocyte adhesion to endothelium or to other immune cells.

In this study, we investigated the role of selectins and integrins as receptors for \( E. \) chafeensis entry into the macrophage, and demonstrated that attachment of \( E. \) chafeensis to a human monocyte cell line THP-1 is mediated in part by cell surface proteins E-selectin and L-selectin.

2. Materials and methods

2.1. Preparation of host cell-free \( E. \) chafeensis stock

DH82 cells were cultivated with 5% bovine calf serum-supplemented minimal essential medium (MEM) at 37°C and used for propagation of \( E. \) chafeensis. DH82 cells were inoculated with \( E. \) chafeensis Arkansas strain when the monolayers were 80% confluent and cultured at 37°C for 4–5 days until 100% of cells were infected with \( E. \) chafeensis. The infected cells were harvested by centrifugation at 12,100 \( \times g \) for 20 min and the resulting pellet was sonicated at 40 W for 10 s three times. The cell lysate was centrifuged at 170,000 \( \times g \) for 10 min and the resulting cell-free \( E. \) chafeensis in the supernatant was centrifuged for 20 min at 12,100 \( \times g \). The pellet was resuspended in freezing medium (10% dimethyl sulfoxide, 20% bovine serum, and 70% MEM). The ehrlichiae were aliquoted and stored at −80°C for infection of THP-1 cells and determination of the \( E. \) chafeensis infectious dose.

2.2. Determination of \( E. \) chafeensis infectious dose

The \( E. \) chafeensis infectious dose was determined by limiting dilution at 10-fold increments of host cell-free \( E. \) chafeensis \( (10^{-1}–10^{-23}) \) and 0.1 ml of each dilution was added to each well of two 24-well plates with DH82 cell monolayers, a canine histiocyte cell line. Cells were incubated at 37°C with 5% \( CO_2 \), and the medium was changed twice weekly. On days 7 and 14 after infection, the infected cell cultures were tested by polymerase chain reaction (PCR) and examined by Diff-Quik staining to monitor \( E. \) chafeensis infection. DNA was extracted from pelleted \( E. \) chafeensis-infected cells using a Qiagen DNA blood Mini Kit (Qiagen, Valencia, CA, USA), and 1 \( \mu l \) of DNA template was used in a PCR reaction to amplify the \( E. \) chafeensis gp120 gene with primers pxcf3b (CAG CAA GAG CAA GAA GAT GAC) and pxar5 (ATC TTT CTC TAC AAC AAG CGG) [14]. PCR amplification was performed using the following thermal cycling protocol: 30 cycles at 94°C for 40 s, 55°C for 40 s, and 72°C for 1 min with a final extension of 7 min at 72°C. Infected cells were cyt centrifuged onto a slide, stained and examined for the presence of \( E. \) chafeensis morulae using a cytologic staining procedure.

2.3. Identification of cell surface receptors

Monoclonal antibodies (mAbs) to E-selectin (sc-5262), L-selectin (sc-13505), P-selectin (sc-8419), integrin \( \alpha m \) (sc-1186), and integrin \( \alpha 4 \) (sc-1185) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used to block \( E. \) chafeensis attachment to THP-1 and HeLa cells. mAbs were specific for human selectins or integrins, except the mAb to L-selectin, which was raised against the lectin domain of L-selectin of mouse origin, cross-reacting with human L-selectin. THP-1 cells were incubated in Hanks’ balanced salt solution with calcium and 10% human AB serum for 30 min at 37°C before the experiment to block non-specific binding. Treated THP-1 cells (1 × 10^6 cells ml^-1) were incubated for 1 h at room temperature or at 4°C with three concentrations of each mAb (40, 20, and 10 \( \mu g \) ml^-1) to evaluate the dose dependence of blocking of \( E. \) chafeensis attachment. THP-1 cells were incubated with host cell-free \( E. \) chafeensis organisms (100 \( \mu l \)) for 30 min at a multiplicity of infection of 25. Cells were pelleted and washed twice with phosphate-buffered saline (PBS), and 2 ml of fresh MEM with 50 \( \mu g \) ml^-1 of gentamicin was added to each tube to kill extracellular ehrlichiae [15]. One ml of the suspension was removed, and DNA extracted immediately from the pelleted cells for semiquantitative PCR and real time PCR. The remaining cell suspensions were incubated at 37°C with 5% \( CO_2 \), and the cells were pelleted the next day. Supernatants were removed, and cells were resuspended in 1 ml of fresh MEM without gentamicin and incubated at 37°C with 5% \( CO_2 \) for 5 days. The cells were harvested and DNA extracted for semiquantitative PCR and real time PCR. Cells serving as positive controls prior to \( E. \) chafeensis infection were treated with PBS, normal control mouse IgG, 0.1% sodium azide, or 0.2% gelatin instead of mAbs. Blocking \( E. \) chafeensis attachment to HeLa cells was performed at room temperature in a similar way as the experiments using THP-1 cells. Briefly, each mAb was used at four concentrations of 5, 10, 20, and 40 \( \mu g \) ml^-1. The experiments were performed in 24-well plates, and non-attached \( E. \) chafeensis were removed by aspiration. Plates were incubated at 37°C with 5% \( CO_2 \) for 5 days.
2.4. Real time PCR and semiquantitative PCR

We assessed *E. chaffeensis* binding to target cells and the development of infection after mAb treatment by real time PCR assay using an iCycler IQ Detection System (Bio-Rad, Hercules, CA, USA) to measure relative differences between samples. The amplification of *E. chaffeensis* DNA was performed in duplicate with the Brilliant Quantitative PCR Core Reagent Kit with SYBR green I reagent (Stratagene, La Jolla, CA, USA) using the following primers: ECF-dsb-262-f (GCA GGA AAT AAA GAC AG) and ECF-dsb-323-r (GCA ATA ACC ACA AGA GT). ECF-dsb-262-f and ECF-dsb-323-r were used to target a disulfide bond formation (Db) protein gene of *E. chaffeensis* [16]. A separate PCR using human G3PDH gene as a target of an internal control for the quantity and quality of host cell DNA was performed on all samples, using the forward primer huG3PDH5 (GTC ATC CCT GAG CTG AAC) and reverse primer huG3PDHgv-r (GTC ATC ATA TTT GGC AGG TTT T). The same DNA samples used in the semiquantitative PCR from mAb blocking experiments were used in real time PCR. One µl of each DNA sample was used as template. The protocol for real time PCR included a cycle of 9 min at 95°C and 40 cycles of 15 s at 95°C, 30 s at 50°C and 20 s at 72°C. Data were acquired during the annealing step of the reaction. Relative DNA levels were determined by subtracting the difference of the Ct (cycle threshold) levels between the target *E. chaffeensis* gene and the host cell housekeeping gene as calculated by target gene Ct and housekeeping gene as calculated by target gene Ct and housekeeping gene Ct. After the data were normalized, the efficiency of binding to and infection of the host cell by *E. chaffeensis* was evaluated.

For semiquantitative PCR, DNA was extracted from THP-1 and HeLa cells (DNA blood Mini Kit), and the DNA concentrations determined by the PicoGreen ds DNA Quantitation Kit (Molecular Probes, Eugene, OR, USA) and adjusted to the same amount in all samples. DNA from each sample was amplified using a duplex PCR with primer pair pxcf3b and pxar5 specific for the *E. chaffeensis* gp120, and primers HuG3PDH5’ (TGA AGG TCG GAG TCA ACG GAT TTG GT) and huG3PDH3’ (CAT GTG GCC CAT GAG GTC CAC) specific for the human G3PDH gene, a housekeeping gene. The housekeeping gene was used to indicate that equal amounts of ehrlichial DNAs were used in each experiment because the amount of ehrlichial DNA in the samples is insignificant compared to the host DNA. DNA template (1 µl) diluted 2, 4, 8, 16, 32, and 64 times was used as template for PCR. PCR reactions were carried out using the Roche PCR Master Kit (Roche Biochemicals, Indianapolis, IN, USA) including 5 pmol of the PCR primers and 1 µl of the extracted DNA samples. These reactions were incubated at 94°C for 5 min; then 30 cycles of the following: 40 s at 94°C, 40 s at 55°C, 1 min at 72°C; and a final elongation step for 7 min at 72°C. The reaction without sample DNA was used as negative control. Ten µl of each sample was electrophoresed through a 1.2% agarose gel to confirm the expected size. Semiquantitative PCR was used to quantitate the amount of *E. chaffeensis* attached to THP-1 or HeLa cells. The PCR titer referred to the last dilution of the template from which a positive PCR result was observed. The inhibition rate of *E. chaffeensis* attachment to cells was calculated by the equation [(PCR titer of PBS-treated cells minus PCR titer of sample treated with mAb)/PCR titer of PBS-treated cells × 100].

2.5. Detection of *E. chaffeensis* morulae

*E. chaffeensis*-infected cells were examined 5 days after inoculation. The *E. chaffeensis* infection rate was determined by evaluating 100 cells. The statistical difference in infection rate was compared among experimental groups using two-tailed t-tests and χ² analysis, utilizing Microsoft Excel software.

2.6. Flow cytometric assay

THP-1 (2 × 10⁶) and HeLa cells (2 × 10⁶) were harvested and washed twice with cold PBS by centrifugation at 300 × g for 5 min. Cells were resuspended in 0.5 ml cold PBS containing one of the mouse mAbs to E-selectin, L-selectin, P-selectin, integrin αm, and integrin αζ at a final concentration of 0.4 µg ml⁻¹ and incubated for 1 h at room temperature. The cells were washed and incubated with a fluorescein-conjugated goat anti-mouse antibody (1:1000) for 30 min in the dark at room temperature. The cells were washed three times and resuspended in 0.5 ml of cold PBS. The resuspended cells were transferred to culture test tubes (12 × 75 mm) and then analyzed on a Becton Dickinson FACScan (BD Biosciences, Franklin Lakes, NJ, USA).

2.7. RNA isolation and cDNA synthesis

To determine the expression of E-selectin, L-selectin, and P-selectin genes in THP-1 cells, reverse transcription (RT)-PCR was performed. Total RNA was isolated from THP-1 cells using the NucleoSpin RNA and Virus Purification kits (BD Biosciences Clontech). The purity of RNA was determined by the PicoGreen RNA Quantitation Kit (Molecular Probes) and the quality of the RNA was verified by agarose gel electrophoresis. Total cellular RNA (2 µg) was used for first strand cDNA synthesis using the cDNA Cycle Kit (Invitrogen, Carlsbad, CA, USA) for RT-PCR. PCR reactions were carried out with the Roche PCR Master Kit (Roche Biochemicals). E-selectin-specific primers were Ef (CCG AAG GGT TTG GTG AGG TGT GCT) and Er (GAA ATG GTG CTA ATG TCA GGA GGG AGA GTC). L-selectin primers were Lf (GTC ATC CCT GAG CTG AAC) and reverse primer huG3PDHgv-r (GTC ATC ATA TTT GGC AGG TTT T).
3. Results

3.1. Inhibition of *E. chaffeensis* attachment by mAbs

The results of two independent experiments were consistent. The number of *ehrlichiae* in THP-1 cells treated with mAbs or control reagents prior to inoculation of *E. chaffeensis*. After removing unbound *E. chaffeensis*, DNA of infected cells were extracted and amplified by PCR for the *E. chaffeensis* gp120 gene. PCR amplification of the housekeeping gene, G3PDH, from the same samples was used as a negative control. Amplicons with expected sizes were sequenced.

and integrin αm were similar to that of the PBS control as determined by semiquantitative PCR. However, the quantity of *ehrlichiae* in samples derived from THP-1 cells treated with mAbs to L-selectin and E-selectin were lower than those of samples treated with PBS at room temperature or 4°C. The decrease in quantity of *E. chaffeensis* in THP-1 cells treated with antibodies to E-selectin and L-selectin at room temperature was 50% and 75%, at antibody concentrations of 10 and 20 μg ml⁻¹, respectively (Fig. 1). The quantity of *E. chaffeensis* in THP-1 cells treated with antibodies to E-selectin and L-selectin at 4°C decreased 50% at antibody concentrations of 10 and 20 μg ml⁻¹ (data not shown). There was no difference in the ehrlichial burden between HeLa cells treated with mAbs to any selectin or integrins and the HeLa cells in the control group (Fig. 1). PCR using huG3PDH primers

Fig. 1. PCR amplification of the *E. chaffeensis* gp120 gene to quantitate *E. chaffeensis* attachment to THP-1 cells. THP-1 cells were treated with mAbs or control reagents prior to inoculation of *E. chaffeensis*. After removing unbound *E. chaffeensis*, DNA of infected cells were extracted and amplified by PCR for the *E. chaffeensis* gp120 gene. PCR amplification of the housekeeping gene, G3PDH, from the same samples was used as a negative control. Amplicons with expected sizes were sequenced.

Fig. 2. Real time PCR amplification of the *E. chaffeensis* dsb gene to quantitate *E. chaffeensis* attached to THP-1 cells that had been treated with mAbs or control reagents.

Fig. 3. Inhibition of *E. chaffeensis* infection in THP-1 cells by mAbs. THP-1 cells were treated the same as described in Fig. 1, but the cells were incubated at 37°C for 5 days after removing the unbound *E. chaffeensis*. A: Prior to inoculation of *E. chaffeensis* THP-1 cells were incubated with mAbs to L-selectin, E-selectin, P-selectin, integrin αm, integrin αx. The controls were THP-1 cells not infected with *E. chaffeensis* (negative) or treated with PBS (positive) and normal mouse IgG. The concentrations of the antibodies were 20 μg ml⁻¹. B: Dose dependence of inhibition of *E. chaffeensis* infection by mAb to E-selectin. C: Dose-dependent inhibition of *E. chaffeensis* infection by mAb to L-selectin.
amplified DNA from all samples at similar dilutions (Fig. 1), indicating that the samples had similar amounts of template copies.

Real time PCR results were consistent with results obtained by semiquantitative PCR. Cts of samples treated with E-selectin and L-selectin mAbs were higher than the Cts of those samples treated with P-selectin, integrin αm, integrin αx and control groups (Fig. 2).

3.2. Inhibition of *E. chaffeensis* infection by mAbs

*E. chaffeensis*-infected THP-1 cells that were treated with mAbs or control agents prior to *E. chaffeensis* infection were evaluated 5 days post inoculation by PCR. The THP-1 cells treated with normal mouse IgG, mAbs to P-selectin, integrin αx, integrin αm or PBS control had similar ehrlichial burdens using a range of mAb concentrations (10–40 µg), whereas the ehrlichial burden was decreased 75–87.5% when THP-1 cells were treated with mAbs to E-selectin or L-selectin compared to the PBS control depending on the concentration of the mAbs (Fig. 3). The ehrlichial burden was decreased 50% when THP-1 cells were treated with E- or L-selectin at 4°C (data not shown). There was no difference in the ehrlichial burden between HeLa cells treated with mAbs to any selectin or integrin compared to the control group (data not shown). All samples amplified with huG3PDH primers had similar ehrlichial burdens, indicating that all samples had similar amounts of template copies.

Results obtained with real time PCR were consistent with those obtained with the semiquantitative PCR method. The Ct values of samples treated with E-selectin and L-selectin mAbs were lower than the Ct values with mAbs against P-selectin, integrin αm, integrin αx and the control groups (data not shown).

Five days after inoculation, the *E. chaffeensis* infection in THP-1 cells was evaluated by light microscopy using Diff-Quik-stained cells prepared by cytospins. In three experiments, only 1–2% of THP-1 cells treated with mAbs to L-selectin or E-selectin contained small numbers of morulae, whereas 30–80% of THP-1 cells treated with controls or mAbs to P-selectin, integrin αx and integrin contained ehrlichial morulae (Fig. 4, *P* < 0.001). None of the mAbs used in the experiment had any effect on *E. chaffeensis* infection in HeLa cells (data not shown).

After demonstrating that E-selectin and L-selectin partially blocked the attachment of *E. chaffeensis* Arkansas strain to THP-1 cells, we evaluated blocking of the attach-

![Fig. 4. Diff-Quik staining of THP-1 cells 5 days post inoculation with *E. chaffeensis*. The cells used in these experiments were treated the same as those in Fig. 3.](image-url)
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Fig. 5. Flow cytometric analysis of the cellular markers on THP-1 cells including E-selectin, L-selectin, P-selectin, integrin αm and integrin αx. Normal mouse IgG and PBS were used as controls. The right shift of the fluorescence peak in THP-1 cells reactive with mAbs to E-selectin or L-selectin indicated the presence of E-selectin and L-selectin on THP-1 cells respectively.

3.3. Detection of L- and E-selectin on THP-1 cells

Flow cytometry confirmed that THP-1 cells expressed L-selectin and E-selectin, but the presence of other antigens (P-selectin, integrin αm and integrin αx) was not observed (Fig. 5). None of these antigens was found on HeLa cells as determined by flow cytometry (data not shown).

DNA fragments with expected sizes were amplified consistently by RT-PCR from THP-1 cells using primers derived from L-selectin and E-selectin genes and were amplified only once using P-selectin gene primers. BLAST analysis showed that the RT-PCR products were similar to the mRNA sequences of human L-selectin, E-selectin, and P-selectin, respectively (data not shown). The mRNA sequences of L-selectin, E-selectin, and P-selectin were deposited in GenBank (accession numbers AY367061, AY367062, AY367063, respectively).

4. Discussion

As *E. chaffeensis* is an obligatory intracellular bacterium, attachment to the host cell surface is crucial for it to enter the host cell and establish infection. The tropism of *E. chaffeensis* for monocytes suggests that this organism uses a specific receptor-mediated pathway for cellular adhesion and entry. *E. chaffeensis* cultured in vitro infects a variety of cells including endothelium, fibroblasts, and epithelium [17,18]. However, in vivo *E. chaffeensis* only infects human monocytes. We hypothesized that *E. chaffeensis* may preferentially bind to specific receptors on monocytes and macrophages in vivo; however, *E. chaffeensis* organisms passaged in vitro appear to adapt and perhaps bind to secondary receptors that appear on a variety of cells, which would explain the expanded cell tropism.

We found that mAbs to L- and E-selectin can reduce the attachment and infectivity of *E. chaffeensis* in THP-1 cells at both room temperature and 4°C. The reduced infectivity is most likely caused by inhibition of *E. chaffeensis* attachment to THP-1 cells rather than inhibition of *E. chaffeensis* growth by the antibodies because antibodies to E-selectin and L-selectin had no effect on the growth of *E. chaffeensis* in HeLa cells. The incomplete blocking of *E. chaffeensis* attachment to THP-1 cells by mAbs to E-selectin and L-selectin is most likely due to non-selectin receptors on THP-1 cells. This hypothesis was confirmed in experiments using HeLa cells, which are deficient in surface expression of L- or E-selectin, but support *E. chaffeensis* growth in vitro.

Selectins are cell adhesion molecules critically involved in the regulation of leukocyte traffic. Selectins mediate adhesion of leukocytes to endothelial cells. L-selectin is expressed on essentially all blood neutrophils and monocytes, and on the majority of blood-borne T and B cells [12]. E-selectin is considered to be limited to endothelium and is upregulated in response to inflammatory stimuli such as interleukin-1, tumor necrosis factor-α, or bacterial lipopolysaccharide [12]. P-selectin is considered to be expressed on activated platelets and endothelium and its expression was also inducible [12]. With flow cytometry and RT-PCR, we demonstrated the expression of E- and L-selectins on THP-1 cells, but we failed to demonstrate the expression of E- and L-selectins on HeLa cells. These selectins are most likely expressed constitutively in THP-1 cells since three experiments detected their expression in THP-1 cells at different time points.

We tried to address whether P-selectin was involved in *E. chaffeensis* attachment to host cells. Our results indicated that antibodies to P-selectin have no effect on *E. chaffeensis* attachment to THP-1 cells. We further investigated the expression of P-selectin on THP-1 cells. With RT-PCR, P-selectin was not consistently detected. Furthermore, flow cytometry also failed to detect P-selectin on THP-1 cells. These results indicated that P-selectin was expressed transiently in THP-1 cells. Our results also indicate that antibodies to integrins fail to block *E. chaffeensis* attachment to THP-1 cells. Since the THP-1 cell is a human macrophage cell line, the role of L- and E-selectins...
tins as the receptors for *E. chaffeensis* needs to be further investigated. The receptor of *E. chaffeensis* may localize in the lectin domain of L-selectin since the mAbs to L-selectin were raised against the lectin domain of L-selectin. Identification of the receptor will advance the development of pharmaceuticals to block the receptor-adhesin interaction for treatment of ehrlichial infections.

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References


