Antigenic Diversity of Granulocytic *Ehrlichia* Isolates from Humans in Wisconsin and New York and a Horse in California

Kristin M. Asanovich, Johan S. Bakken, John E. Madigan, Maria Aguero-Rosenfeld, Gary P. Wormser, and J. Stephen Dumler

Division of Medical Microbiology, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland; Section of Infectious Diseases, Duluth Clinic, Duluth, Minnesota; Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California; Department of Pathology and Division of Infectious Diseases, Department of Medicine, New York College of Medicine and Westchester County Medical Center, Valhalla, New York

The agent of human granulocytic ehrlichiosis (HGE), *Ehrlichia phagocytophila,* and *Ehrlichia equi* are very similar. HGE is of variable severity. Genetic and antigenic differences among 3 human isolates (Webster, Spooner, and NY-8) and 1 horse isolate (MRK) were evaluated. The 16S rRNA gene sequences were identical in all human isolates. By use of 5 homologous antisera from these 3 humans and 1 horse and an additional 5 antisera in heterologous reactions, the immunodominant antigens of each isolate were noted to differ in molecular size: 43 kDa in the Webster (Wisconsin) isolate, 46 kDa in the Spooner (Wisconsin) isolate, 42 and 45 kDa in the NY-8 (New York State) isolate, and a 42 kDa doublet in the *E. equi* MRK isolate from California. Two sera from a Wisconsin patient reacted weakly or not at all with the NY-8 isolate. Antigenic structural diversity exists among otherwise indistinguishable granulocytic ehrlichial isolates.

The recent recognition of human granulocytic ehrlichiosis (HGE), an illness caused by an agent genetically and morphologically similar to *Ehrlichia phagocytophila* and *Ehrlichia equi,* has helped to explain some of the undiagnosed illnesses that occur after tick bites in the upper Midwest, northeastern, and Pacific states in the United States [1–4]. The spectrum of disease attributed to infection with the HGE agent varies from clinically inapparent to severe or fatal infections [3, 5–8]. Whether variability in severity of illness results from host or bacterial factors, or both, is not known.

Until recently, the relationships among some named species of the genus *Ehrlichia* were not well defined and were based in part on geography, host species, infected host cell, and morphology. Use of 16S rRNA gene phylogenetic analysis by Anderson et al. [9] showed that *E. equi* and *E. phagocytophila* have nearly identical sequences and suggested that these two separately designated species may actually be the same. Convalescent sera from patients with HGE, dogs with *''E. equi''* infection, *E. equi*–infected horses, and *E. phagocytophila*–infected cattle from Europe contain antibodies that react with a major 44-kDa *E. equi* antigen not detected by antibodies to other *Ehrlichia* species, proving that these ''species'' also share significant antigenic components as well [10]. The biologic identity of *E. equi* and the agent of HGE was further confirmed when blood from a symptomatic human patient transfused into a horse was able to cause typical equine granulocytic ehrlichiosis and induced protective immunity to challenge with *E. equi* in convalescence [11, 12]. Because of these similarities, the closely related ehrlichiae, *E. equi, E. phagocytophila,* and the HGE agent are grouped within a cluster called the *E. phagocytophila* (Ep) genogroup [3, 4].

Recent developments with in vitro propagation have afforded the opportunity to study the molecular, antigenic, and cellular components of Ep group ehrlichiae [13, 14]. Cultivation of Ep group ehrlichiae in HL60 promyelocyte cells was first reported in 1995, and several human isolates have subsequently been cultivated from different geographic regions where HGE has been reported [13, 15, 16]. Thus, the propagation of these isolates allows study of the differences between individual isolates and the relationship of specific molecular and antigenic components to pathogenicity. We wished to characterize the potential genetic and antigenic differences in pathogenic Ep group ehrlichiae collected from different geographic regions. Thus, we studied the 16S rRNA gene sequences and the immunoblotted antigens in isolates from 3 humans and 1 horse by reacting all antigens with homologous and heterologous sera from convalescent patients and experimentally infected horses.

Materials and Methods

Polymerase chain reaction (PCR) and isolation of HGE agents and E. equi by tissue culture. Acute-phase blood, drawn into

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Reprints or correspondence: Dr. J. Stephen Dumler, Division of Medical Microbiology, Dept. of Pathology, Johns Hopkins Medical Institutions, Meyer B1-193, 600 North Wolfe St., Baltimore, MD 21287.

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Table 1. Patient blood used for culture and sera used to detect immunoreactive antigens of *E. phagocytophila* group ehrlichial isolates from 3 humans and 1 horse.

	Acute-phase blood			Convalescent serum	
Sample, location	Day after onset of illness	% infected leukocytes	PCR result	Day after onset of illness	*E. equi* IFA titer
Webster isolate, Wisconsin	3	1.4	+	35	1280
Spooner isolate, Wisconsin	1	0.4	+	11	1280
				67	320
NY-8 isolate, New York	17	0.3	+	26	⩾2560
E. equi MRK strain, California	13*	50	+	30*	⩾2560
HGE agent BDS strain, Wisconsin	NU	NU	NU	30*	⩾2560
Patient 4, New Jersey	NU	NU	NU	27	1280
				54	640
Patient 5, Wisconsin	NU	NU	NU	11	⩾2560

NOTE. PCR, polymerase chain reaction; NU, not used.
* Day after experimental inoculation with *E. equi*.

EDTA-treated tubes, was obtained from 3 patients with suspected HGE and from 1 horse experimentally infected with *E. equi* MRK (table 1). Each patient and the horse had typical ehrlichial morulae in peripheral blood neutrophils at the time the blood was obtained. Two patients resided in northwest Wisconsin; another resided in Westchester County, New York, and will be reported elsewhere (Wormser GP, unpublished data). The MRK strain of *E. equi* was initially isolated from a naturally infected horse from northern California [10]. To prove that the patients and the horse were actively infected, PCR for the Ep group 16S rRNA gene was performed on each blood sample using the Ep group-specific primers ge9f and ge10r, as previously described [17]. In addition, indirect IFA serology on acute and convalescent paired sera for each of 5 patients (total, 4 acute and 6 convalescent sera obtained 11–67 days after onset of illness) and 2 horses (2 preinoculation sera and 2 30-day postinoculation sera) (see below, Sera for Immunoblotting) showed at least a 4-fold increase in Ep group antibody titer during convalescence using *E. equi* as antigen.

EDTA-anticoagulated blood (100 μL) was inoculated into 5 mL of HL60 promyelocyte cell line in RPMI 1640 supplemented with 2 m*M* L-glutamine and 1% fetal bovine serum at a final concentration of 2×10^5 cells/mL. The cultures were maintained in a 5% CO_2 incubation chamber at 37°C and examined at least every 3 days by Romanowsky staining (LeukoStat; Fisher Scientific, Pittsburgh, PA) of cytocentrifuged cells for the presence of characteristic morulae. When observed, morulae were confirmed to be ehrlichiae in the Ep group by IFA using horse anti–*E. equi* or human anti–HGE agent sera as described [10]. Once infection was established (⩾90% of cells infected), the ehrlichiae were propagated by subsequent passage onto uninfected HL60 cells. The identity of each isolate was confirmed by PCR amplification of the 16S rRNA, followed by sequence analysis.

DNA sequencing. Total host cell and ehrlichial DNA from infected HL60 cells was purified (Puregene DNA isolation kit; Gentra Systems, Minneapolis). The 16S rRNA genes were amplified by PCR as previously described [1, 18] using universal eubacterial primers that anneal to conserved regions on the 5′ and 3′ ends to produce an ~1434-bp fragment constituting >95% of the 16S rRNA gene. The success of the amplification was monitored by separating the amplified products by electrophoresis in a 1% agarose gel and visualizing bands of appropriate molecular size with ethidium bromide. Water and uninfected HL60 cells were used as negative controls. Products of PCR were sequenced using a fluorescent automated sequencer and a cycle sequencing protocol (model 377; Applied Biosystems, Foster City, CA).

Purification of ehrlichiae from host cells. The isolates were propagated continuously for 103 days (13 passages) for the Webster isolate (northwest Wisconsin), 103 days (7 passages) for the Spooner isolate (northwest Wisconsin), 23 days (4 passages) for the NY-8 isolate (southeast New York State), and 39 days (4 passages) for the *E. equi* MRK isolate (northern California). When a total of ~$1.5–6.0 \times 10^8$ infected cells was available in the cultures, ehrlichiae were separated from host cells through a modified density gradient centrifugation procedure using diatrizoate meglumine [10, 19]. Briefly, cells and bacteria were centrifuged for 15 min at 28,000 *g* then resuspended in 0.01 *M* phosphate buffer containing 218 m*M* sucrose and 5 m*M* L-glutamine (SPGN) [20]. The infected cells were lysed by sonication on ice, layered onto a 19% diatrizoate meglumine cushion, and centrifuged at 27,000 *g* for 1 h at 4°C. The ehrlichial pellet was resuspended in SPGN with 50 mg/mL RNase and 50 mg/mL DNase for 45 min and incubated in a 37°C water bath.

The ehrlichial preparation was dispensed onto a discontinuous diatrizoate meglumine gradient consisting of layers with 45%, 30% and 19% diatrizoate meglumine and centrifuged at 27,000 *g* for 1 h at 4°C. The interface between the 30% and 19% layers (T band), and between the 45% and 30% layers (H+L band) were initially harvested and assessed for ehrlichial yield and purity by light microscopy. Thereafter, the layer between the 45% and 30% diatrizoate meglumine layers was harvested and washed in SPGN to remove residual diatrizoate meglumine. The ehrlichial pellet was resuspended in 2–5 mL of SPGN, and the protein concentration was measured (BCA protein assay reagent; Pierce, Rockford, IL). Density gradient–purified ehrlichial stocks were frozen at −80°C in SPGN until used.

Uninfected control cell antigens were prepared by harvest of cells and sonication as described above. However, since no material was present at the interfaces after density gradient centrifuga-

tion of lysed uninfected cells, the sonicated cells were used directly as immunoblot antigen after quantitation of protein content.

SDS-PAGE of ehrlichiae. SDS-PAGE and immunoblotting were performed as described [10, 21]. Frozen stocks of purified ehrlichiae were rapidly thawed at 37°C in a water bath and then centrifuged in a microcentrifuge at 18,000 *g* for 1 min to pellet the ehrlichiae. The pellet was resuspended in SDS-PAGE sample buffer (final concentration: 32 m*M* TRIS-HCl, 5 m*M* EDTA, 13.8% 2-mercaptoethanol, 3.6% saturated bromophenol blue solution, 36% glycerol) to a concentration of 2, 4, or 6 mg of protein/mL (determined by BCA protein assay; Pierce). Samples and prestained protein standard were heated at 95°C for 5 min. Sample (25 μL of each, separately) was loaded into lanes of an 18 × 20 × 15 cm polyacrylamide gel consisting of a 2.5% stacking layer and a 12.5% separating layer. Electrophoresis was continued overnight at 50 V, until the dye front was eluted from the gel. Gels were used for electrotransfer to nitrocellulose for immunoblotting.

Immunoblotting. Ehrlichial proteins that were separated by SDS-PAGE were transferred to a 15 × 15 cm nitrocellulose paper at 1 A for 2 h at 4°C [22]. After electrotransfer, the blots were carefully labeled, air-dried for 20 min, and stored at −20°C in airtight plastic bags until needed. Immunoblots were brought to room temperature, and nonspecific protein binding was blocked by preincubation in 0.1 *M* PBS with 1% nonfat dry milk, 0.05% Tween 20, and 1% normal goat serum (PBSTNG) for 10 min at room temperature. The immunoblots were then incubated at room temperature for 2 h with appropriate sera diluted at 1:80 in PBSTNG. The immunoblots were washed three times with PBS with 0.05% Tween 20 for 10 min each wash and then incubated for 1 h with the appropriate secondary antibody, either alkaline phosphatase–labeled goat anti–human IgG, γ-chain specific, or alkaline phosphatase–labeled goat anti–horse IgG, γ-chain specific (Kierkegaard & Perry Laboratories, Gaithersburg, MD) diluted 1:5000 and 1:500 in PBSTNG, respectively, per the manufacturer's instructions. After another wash, bound antibodies were visualized with BCIP/NBT (5-bromo-4 chloro-3-indolyl phosphate at 170 μg/mL with nitroblue tetrazolium at 330 μg/mL in 100 m*M* TRIS-HCl, pH 9.5, 100 m*M* NaCl, and 5 m*M* $MgCl_2$ alkaline phosphate substrate buffer; Sigma, St. Louis). The immunoblots were rinsed with deionized water and air-dried for 15 min. The molecular size of the visualized bands was determined by comparison with the molecular size protein standards (prestained high molecular weight protein standard, GIBCO-BRL, Gaithersburg, MD).

Sera for immunoblotting. The characteristics of the 7 human and 2 horse sera reactive with *E. equi* by IFA that were used in the immunoblotting methods are shown in table 1. All sera were found nonreactive by IFA for antibodies to *Ehrlichia chaffeensis.* After onset of illness, convalescent human sera were obtained at 35 days for the Webster isolate, 11 and 67 days for the Spooner isolate, and 26 days for the NY-8 isolate. Additional convalescent sera were also obtained 11 days after onset of illness in a patient with HGE who resided in northwestern Wisconsin (proven by PCR) and 27 and 54 days after onset of illness in a patient from northern New Jersey (proven by seroconversion). Human control sera were obtained from laboratory personnel who had not worked with ehrlichiae before and had no history of ehrlichiosis. Horse serum was obtained from animals 30 days after experimental infection with *E. equi* (MRK strain) or with the HGE agent (BDS strain) and from a normal *E. equi*-IFA–seronegative horse with no history of equine granulocytic ehrlichiosis [11].

Each serum was reacted with each antigen preparation separated in a single polyacrylamide gel under identical conditions and transferred onto a single nitrocellulose paper. Each of these nitrocellulose papers was reacted with a single diluted serum, so that reactions for each antigen with each serum were under identical conditions.

Results

Peripheral blood examination and PCR. Examination of Wright-stained peripheral blood smears revealed that the 3 patients had ehrlichial morulae in 1.4%, 0.4%, and 0.3% of total leukocytes. PCR analysis of acute-phase peripheral blood at the time of tissue culture inoculation revealed Ep group 16S rRNA genes in each sample (data not shown). Peripheral blood from the horse experimentally infected with *E. equi* was obtained at a time when ~50% of the neutrophils contained ehrlichial morulae (13 days after inoculation). PCR also showed the presence of Ep group DNA in the horse acute-phase peripheral blood (data not shown).

Sequence analyses of the 16S rRNA genes of the human Ep group isolates. The 16S rRNA gene sequence of each human Ep group isolate was identical to the published sequence for the HGE agent (GenBank U02521) initially identified in a patient from Spooner, Wisconsin. The nucleotide sequence for *E. equi* MRK has been published and is 99.9% identical to that of the HGE agent and *E. equi* (GenBank M73223) originally derived from a California horse [1, 9, 13].

Isolation and density gradient purification of ehrlichiae. Ehrlichiae were first detected in HL60 tissue culture cells by Romanowsky and fluorescent antibody staining between 5 and 11 days after inoculation with infected blood. Romanowsky stains detected infected cells in each culture but underestimated the percentage of tissue culture cells infected when compared with fluorescent antibody stains. Thus, the percentage of infected cells in each culture was determined definitively by fluorescent antibody staining.

Each isolate was continuously propagated by addition of uninfected HL60 cells until sufficient numbers of heavily infected cells were observed (>90% of cells containing morulae by IFA). Each preparation of density gradient–purified ehrlichiae was found to be free of extraneous bacteria and fungi by cultivation on a variety of solid and liquid acellular media. The discontinuous density gradient centrifugation process yielded two distinct bands that contained ehrlichiae, one between the 19% and 30% diatrizoate meglumine layers (T) and one between the 30% and 45% diatrizoate meglumine layers (H+L). The H+L band was enriched with intact ehrlichiae, contained less amorphous contaminating material, and was used in all subsequent SDS-PAGE and immunoblots.

Immunoblotting. The most appropriate protein concentration for immunoblotting was determined by empiric dilutions

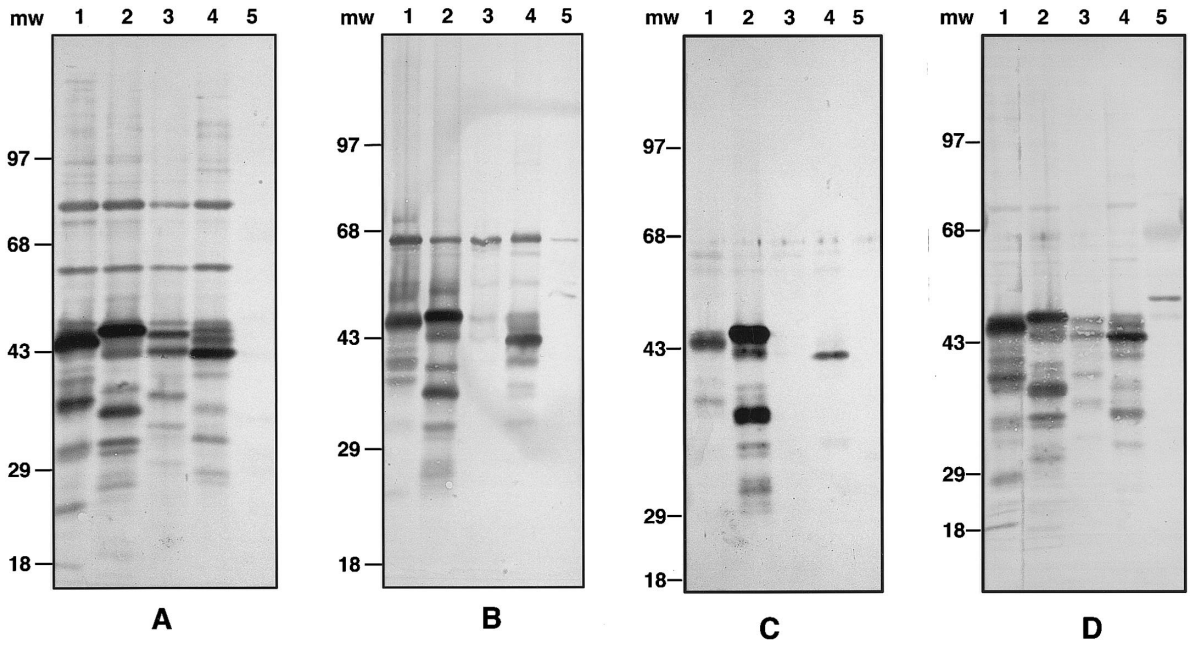

Figure 1. Immunoblots using tissue culture–propagated human granulocytic ehrlichiosis (HGE) agent and *E. equi* isolates as antigens purified by diatrizoate meglumine density gradient. Lanes 1–3, HGE agent isolates Webster (Wisconsin), Spooner (Wisconsin), NY-8 (Westchester County, New York), respectively; lane 4, MRK isolate of *E. equi* (California); lane 5, uninfected HL60 cell lysate. Blots were reacted with, **A**, convalescent serum taken from horse experimentally infected with MRK isolate of *E. equi*; **B**, convalescent serum obtained 35 days after onset of illness from patient infected with Webster isolate; **C**, convalescent serum obtained 14 days after onset of illness from patient infected with Spooner isolate; and **D**, convalescent serum obtained 26 days after onset of illness from patient infected with NY-8 isolate. Molecular sizes are indicated; 200-kDa standard is not shown.

based on protein content of the purified ehrlichiae. Generally, a concentration of ehrlichial protein of 2 mg/mL revealed a large number of bands when reacted with sera of different sources without protein overloading or excessive reactivity. All but 1 convalescent sera from patients diagnosed with HGE and all sera from horses experimentally infected with *E. equi* or the HGE agent (BDS strain) reacted by immunoblot (figure 1); no control human sera or horse sera reacted (data not shown). The Webster and Spooner isolates (Wisconsin) and the *E. equi* MRK (California) reacted with similar immunoblot densities with the same sera; however, the NY-8 isolate (New York) reacted less well with all sera, even when three times the ehrlichial protein concentration (6 mg/mL) was loaded onto the polyacrylamide gels (data not shown). Experiments with varied serum dilutions to assess the potential for antibody interference or blocking in the immunoblots failed to show any significant change in band patterns in reactive samples and occasionally resulted in the appearance of bands in nonimmune sera at dilutions of 1:10 to 1:40.

Representative results of immunoblots are shown in figure 1. Among all of these isolates, 41 total distinct antigen bands ranging in molecular size from 17 to 160 kDa could be detected in side-by-side comparisons. The most intensely stained antigens varied among the isolates but were typically located between 37 and 46 kDa, similar to what was reported for *E. equi* harvested from equid peripheral blood neutrophils [10].

Antigens of 42, 53, 54, 58, 60, 65, and 74 kDa were present in all of the isolates; however, among these, only the 58-, 60-, and 74-kDa bands were frequently detected. High-molecular-size antigens (106, 140, 150, 155, and 160 kDa) were noted in the isolates, although none was common among all isolates. Other observed bands that were not uniformly present in all isolates included antigens of 32, 33, and 34 kDa. The immunodominant antigen profile that was detected with these sera was distinctive for each isolate and reproducible regardless of the serum used (figure 1).

The specific profiles of antigens that typically were detected with the strongest intensity in each isolate are shown in table 2. There were 25, 18, 15, and 16 recognizable bands of at least moderate intensity detected by at least 2 different convalescent sera in the Webster, Spooner, NY-8, and MRK isolates, respectively. The most immunodominant antigens had bands between 37 and 48 kDa but varied for each isolate. Of interest, the 11-day convalescent serum from one Wisconsin patient (Spooner) did not react with any of the antigens of the NY-8 isolate, and a subsequent serum sample obtained at 67 days reacted only minimally with the 42- and 45-kDa immunodominant antigens that were otherwise intensely stained by the 26-day homologous patient convalescent serum. Likewise, the 35-day convalescent serum sample obtained from the other Wisconsin patient (Webster) reacted only weakly with both these NY-8 isolate antigens as well.

Table 2. Antigenic profiles of 3 human granulocytic ehrlichial isolates from Wisconsin and New York and a California *E. equi* isolate from an experimentally infected horse.

Granulocytic *Ehrlichia* isolate	Antigens (kDa)*
Webster (WI) isolate	33, 34, **38**, 40, 42, **43**,† **46**, 48, 58, 60, 65, 74
Spooner (WI) isolate	33, 34, **37**, 39, **42**, **46**,† 58, 60, 65, 74
NY-8 (NY) isolate	36, 38, **42**,† **45**,† 48, 58, 60, 65, 74
E. equi MRK (CA) isolate	34, 41, **42 (doublet)**,† **43**, **46**, 48, 58, 60, 65, 74

* Antigen bands that had at least moderately intense reactions in at least one of 9 sera tested are shown. Antigen molecular sizes in bold type indicate major immunodominant antigens.

† Antigen bands of each isolate that show strongest immunoreactivity with all sera tested.

Most sera had only minimal (fewer than three bands) or no reactions with the uninfected HL60 cell lysate antigen. When observed, these reactions did not correspond to antigens of the same molecular size in the purified ehrlichiae except in 1 sample. Antigens of uninfected HL60 cells that reacted with the sera included antigens of the following molecular sizes: 50 kDa (2 sera), 54 kDa (1 serum), 59 kDa (1 serum), 64 kDa (1 serum), 66 kDa (4 sera), 76 kDa (1 serum), and 100 kDa (1 serum) (data not shown).

Sera from the horses experimentally infected with *E. equi* MRK and the HGE agent BDS isolate routinely reacted with more antigens of each isolate than did convalescent human sera. Neither of these equid sera detected additional major antigen bands not also detected by the convalescent human sera. Normal horse serum and serum from a human subject with no history of HGE did not react with any of the antigens of the Ep group human or equid isolates (data not shown).

Sera from 2 other patients diagnosed with HGE in Wisconsin and northern New Jersey were used to confirm the immunoblot profiles of each isolate. The Wisconsin patient sera reacted similarly to the sera from other Wisconsin patients. However, 2 sera collected at 1 and 2 months after onset of HGE from a human patient in northern New Jersey had particularly strong reactions with 58- and 60-kDa antigens of each Ep group isolate, a pattern not seen with any other sera. These sera reacted well with other antigens from all isolates, and no new significant bands became more readily apparent or less easily distinguished with serum obtained at 2 months compared with serum obtained after 1 month.

Discussion

Only recently have members of the Ep genogroup been cultivated in vitro [13–16]. This has provided an opportunity to examine in greater detail these apparently similar bacteria at antigenic, structural, ultrastructural, biologic, and genetic levels. As expected, all the human HGE agent isolates examined in this investigation had identical 16S rRNA gene sequences. Likewise, each isolate behaved similarly in vitro in HL60 promyelocyte cell cultures, and only minor morphologic and ultrastructural differences of unknown significance could be detected (data not shown).

Immunoblot analyses of 3 granulocytic ehrlichial isolates from infected humans in the upper Midwest and northeastern United States and an *E. equi* isolate from a California horse revealed objective and reproducible differences. All infected patients and the horse developed serum antibodies that reacted with *E. equi* by IFA and with *E. equi* and most of the HGE agents by immunoblotting. Immunoblot analyses showed that all of these Ep genogroup isolates possessed some antigens of identical molecular size, particularly those between 55 and 75 kDa, a range known to contain the GroEL heat-shock protein analogues of other ehrlichiae and rickettsiae [23, 24]. The immunodominant antigens of each isolate were found in the range of 37–46 kDa. However, clear structural differences must exist among the immunodominant antigens of each isolate in order to explain the reproducible differences in the antigen molecular sizes and differential antibody reactivities. Moreover, the major antigenic constituents or structure of the major immunodominant antigens must vary, since the most intensely stained bands differed among isolates but were uniform for each isolate regardless of the sera used (table 2).

The NY-8 isolate lacks or is deficient in some antigenic determinants present in the other Ep genogroup isolates. Immunoblots of the NY-8 isolate produced fewer and weaker bands than the Wisconsin isolates or *E. equi* regardless of the serum used, including homologous convalescent serum. Initially, it was suspected that this reaction was the result of a technical problem; however, proteins from each granulocytic ehrlichia isolate were loaded onto gels at identical concentrations, each serum was reacted with each antigen preparation simultaneously on a single nitrocellulose paper, and a 3-fold increase in ehrlichial protein content for the NY-8 isolate failed to achieve a reaction intensity similar to that of the other isolates at the lower protein concentration. Dilution studies of sera reacted with the NY-8 isolate failed to reveal other technical problems, such as antibody interference or blocking.

It is interesting to note that the molecular size of the 42-kDa major immunodominant antigen of the MRK strain of *E. equi* that has been propagated in promyelocyte cultures differs from the 44-kDa antigen observed when *E. equi* MRK was isolated from infected equine neutrophils [10]. The significance of this finding is unclear and may represent a miscalculation in the original molecular size estimation. Other possible explanations include alterations that result from mutations accumulated during continuous or prolonged in vitro propagation or selective expression of different antigens in vivo or during different life stages.

Such antigenic variability is not unique and has been demonstrated in *E. chaffeensis* and *Ehrlichia risticii.* Antigenic diversity is associated with minor diversity in the 16S rRNA gene

sequences for *E. chaffeensis* and significant sequence variation for *E. risticii* [25–27]. Whether the antigenic differences among these Ep genogroup isolates represent simple polymorphisms or modifications that result in different biologic or pathogenetic activities is not known. The similar in vitro growth, morphology, and cytopathic effects do not suggest differences with in vitro virulence related to different antigenic strains. Inspection of the clinical records revealed no clear differences in disease that could be associated with the different isolates (data not shown).

At a minimum, antigenic diversity may lead to aberrations in the serodiagnosis of HGE. At least 1 patient from whom a granulocytic ehrlichia was isolated from blood in September 1995 never developed an *E. equi* antibody response (tested every 3 months up to 1 year) [6]. This situation possibly resulted from early doxycycline therapy, even though other patients treated early in the course of disease develop significant *E. equi* antibody titers [5, 6]. Thus, it is important to investigate further the antigenic diversity of these ehrlichiae in specific geographic regions to ascertain whether local isolates differ significantly and if serologic tests using antigenic variants would provide useful information for diagnosis. Investigation of the structural and molecular variations among these otherwise similar organisms may also yield important insights into the biologic function of ehrlichial proteins and their involvement in the pathogenesis of HGE.

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