ORIGINAL ARTICLES

Immune Serum From Rabbits Infected with *Borrelia burgdorferi* B31 Confers Complete Passive Protection Against Homologous Challenge
Celeste Chong-Cerrillo, PhD; Xiao-Yang Wu, MD; Yi-Ping Wang, MD;
David R. Blanco, PhD; Michael A. Lovett, MD, PhD; and James N. Miller, PhD

Laboratory and Clinical Findings in Tick-Borne Encephalitis—
Discrimination from Neuroborreliosis
R. Kaiser, PD

Detection of *Borrelia* DNA in Circulating Monocytes as Evidence of Persistent Lyme Disease
Ping Wang, MS; Ronald Gartenhaus, MD; Sunil K. Sood, MD;
James DeVoti, PhD; Carol Singer, MD; Gary Dorante, BS; and Eileen Hilton, MD

Erythema Migrans—My Point of View
Edwin J. Masters, MD

Psychiatric Symptomatology Associated with Presumptive Lyme Disease: Clinical Evidence
Héctor R. Battaglia, MD; Guido Alvarez, MD; Augusto Mercau, MD; Marcelo Fay, MD; and Martin Campodónico

PHOTOGRAPHIC SECTION

An International Collection of Ticks: Part I
James L. Occi
Robert L. Lesser, MD, Yale University School of Medicine
Alan B. MacDonald, MD, Franklin Hospital Medical Center
John E. Madigan, DVM, PhD, University of California—Davis
Edwin J. Masters, MD, Regional Primary Care
Pamela A. Paparone, RN, MSN, Atlantic City Medical Center
Philip W. Paparone, DO, Atlantic City Medical Center
Charles S. Pavia, PhD, New York Medical College
Mario T. Philipp, PhD, Tulane University Primate Center
Julie A. Rawlings, MPH, Texas Department of Health
Ronald F. Schell, PhD, University of Wisconsin School of Medicine
Edward M. Schneider, PhD, Veterinary Research Associates
Martin M. Shinedling, PhD, St. Mary’s Medical Center
Terry L. Schulte, PhD, New Jersey State Department of Health
Steven E. Schutzer, MD, University of Medicine and Dentistry of New Jersey—New Jersey Medical School
Tom C. Schwan, PhD, National Institutes of Health, Rocky Mountain Laboratories
Rudolph J. Schrimpf, MD, Medical College of Wisconsin
Francis Stele, MD, PhD, University Medical Center, Slovenia
Irwin T. Vanderhoof, PhD, New York University Stern School of Business
David J. M. Wright, MD, Charing Cross Medical School, Great Britain

ILLUSTRATIVE EDITOR
James H. Katzel, MD
ORIGINAL ARTICLES

Immune Serum from Rabbits Infected with Borrelia burgdorferi B31 Confers Complete Passive Protection Against Homologous Challenge
Celeste Chong-Cerrillo, PhD; Xiao-Yang Wu, MD; Yi-Ping Wang, MD; David R. Blanco, PhD; Michael A. Lovett, MD, PhD; and James N. Miller, PhD

Laboratory and Clinical Findings in Tick-Borne Encephalitis—Discrimination from Neuroborreliosis
R. Kaiser, PD

Detection of Borrelia DNA in Circulating Monocytes as Evidence of Persistent Lyme Disease
Ping Wang, MS; Ronald Gartenhaus, MD; Sunil K. Sood, MD; James DeVoti, PhD; Carol Singer, MD; Gary Dorante, BS; and Eileen Hilton, MD

Erythema Migrans—My Point of View
Edwin J. Masters, MD

Psychiatric Symptomatology Associated with Presumptive Lyme Disease: Clinical Evidence
Héctor R. Battaglia, MD; Guido Alvarez, MD; Augusto Mercau, MD; Marcelo Fay, MD; and Martín Campodónico

PHOTOGRAPHIC SECTION

An International Collection of Ticks: Part I
James L. Occi

Information for Contributors

New Editor-in-Chief

Richard C. Tilton, PhD, is the new Editor-in-Chief of Journal of Spirochetal and Tick-borne Diseases. Dr. Tilton has over four decades of research and publication experience. He is former Editor-in-Chief of the Journal of Clinical Microbiology and has published over 150 scientific articles, 20 books, and 175 abstracts/brief reports. He has served as principal investigator in more than a dozen scientific studies and is board-certified by the American Board of Medical Microbiology.

Dr. Tilton has earned fellowships from the American Academy of Microbiology, Association of Clinical Scientists, and Infectious Disease Society of America. He earned honorable distinctions from the American Society for Microbiology as Foundation National Lecturer in Clinical Microbiology and is the first recipient of the Abbott Award for Research in Rapid Diagnosis.

Dr. Tilton earned his PhD in microbiology from the University of Massachusetts and started his 24-year college teaching career at Boston University School of Medicine and then moved to the University of Connecticut School of Medicine. He left his position as Associate Dean for Graduate Medical Education to establish and serve as President and Chief Scientific Officer of the North American Laboratory Group. Boston Biomedica acquired the firm and renamed it BBI Clinical Laboratories. Dr. Tilton continues to work for BBI, where he serves as Senior Vice President, Science and Technology.
Immune Serum from Rabbits Infected with *Borrelia burgdorferi* B31 Confers Complete Passive Protection Against Homologous Challenge

Celeste Chong-Cerrillo*, PhD; Xiao-Yang Wu†, MD; Yi-Ping Wang‡, MD; David R. Blanco‡, PhD; Michael A. Lovett‡, MD, PhD; and James N. Miller, PhD

ABSTRACT

Further support for a role of humoral immunity in experimental Lyme disease is shown in this study by the demonstration that passive immunization with infection-derived immune rabbit serum protects rabbits against challenge with *Borrelia burgdorferi*. Animals administered immune rabbit serum both before and after intradermal challenge with virulent *B burgdorferi* strain B31 and were protected against development of erythema migrans (EM) lesions, as well as skin and visceral infection. In contrast, animals that were administered immune serum only after challenge were not protected, although EM lesion development was observed to be altered as compared to controls. Serum antibody levels against outer surface protein (Osp)A, OspC, decorin binding protein (Dbp)A, and DbpB from passively immunized rabbits were determined by ELISA. While rabbits receiving immune sera before and after challenge were protected against infection, their antibody levels against OspA were relatively low and antibody levels against OspC, DbpA, and DbpB were comparable to basal sera. These findings demonstrate that in the rabbit model of Lyme disease, passive humoral immunity can confer complete protection only if administered before challenge. The data also suggest that immune rabbit serum antibody directed against antigens other than OspA, OspC, DbpA, and DbpB may contribute to this protection.

Key words: post-challenge, immune serum, erythema migrans, normal rabbit serum

INTRODUCTION

Lyme disease is a worldwide tick-transmitted infection caused by a group of related spirochetes collectively termed *Borrelia burgdorferi* sensu lato, which includes *B burgdorferi* sensu stricto, *B afzelii*, and *B garinii*. Lyme disease is the most common vector-borne disease in the United States and is transmitted to humans by the bite of an infected *Ixodes* or *Amblyomma* tick. In most patients, Lyme disease is characterized by the initial appearance of a rash-like skin lesion, termed erythema migrans (EM). Early and late clinical manifestations include arthritis, neurological manifestations, lymphadenopathy, and cardiitis, which reflects dissemination to visceral targets. Although Lyme disease is rarely fatal, it can be debilitating.

Implicit in the development of an effective vaccine against Lyme disease is a thorough understanding of the pathogenesis and host immune response operative during host-spirochete interaction. The animal model most frequently used in the study of Lyme disease has been the rodent. The rodent represents a permissive host for *B burgdorferi* as chronic infection is a salient feature, similar to what is seen in human infection. The rabbit model of Lyme disease also has unique features relevant to the immunobiology of human *B burgdorferi* infection. It is the only animal model, besides the rhesus monkey, that reproducibly produces EM indistinguishable from that of human disease after intradermal inoculation of *B burgdorferi*, sensu stricto. Most importantly, untreated skin and visceral infection is ultimately cleared, in contrast to the monkey and rodent models, resulting in complete immunity against reinfestation with up to $2 \times 10^7$ organisms. This protection against challenge with cultivated virulent organisms is more than three orders of magnitude greater than that observed in untreated rodent models.
magnitude greater than that afforded by immunization of rabbits with outer surface protein A (OspA), a protein currently used for human vaccination.

In this study, we used serum from rabbits that have developed complete protective immunity following infection to determine whether this protective immunity can be transferred by passive immunization to naive rabbits. In addition, we sought to correlate passive immunity following challenge with serum antibodies directed against the OspA, OspC, decorin binding protein (Dbp)A, and DbpB antigens of B burgdorferi.

MATERIALS AND METHODS

Animals

Adult, male, New Zealand white rabbits ages 6-9 months (Irish Farms, Norco, CA) were housed individually in a temperature-controlled environment ranging from 18-21°C. Prior to intradermal (ID) inoculation with B burgdorferi, the backs of the rabbits were clipped closely with an electric clipper fitted with a size 40 blade to expose the skin (Oster Professional Products, McMinnville, TN).

Bacterial Strains and Preparation of Challenge Inoculum

Virulent Borrelia burgdorferi sensu stricto, strain B31, was isolated from infected rabbit tissue, grown in BSK II medium to maximum density, then passed twice more in fresh BSK II medium. After the final passage (passage 2), the organisms were centrifuged at 8,000 × g for 10 minutes and washed three times in heat-inactivated (56°C, 30 minutes) normal rabbit serum (NRS) diluted 1:1 with phosphate buffered saline (PBS), pH 7.2 (NRS-PBS). Inoculum for challenge was resuspended in NRS-PBS after the final wash to a final concentration containing 1 × 10⁷ B burgdorferi per mL.

Immune and Nonimmune Sera

As a source of immune serum, 25 rabbits were initially infected ID with 6 × 10⁶ B burgdorferi, B31, allowed to sit for 24 weeks, and then challenged ID with 1 × 10⁷ B burgdorferi, B31. The animals were bleed two weeks later, at a time when they were shown, by culture of their skin and viscera, to be immune. We have established previously that after 24 weeks, rabbits resolve both local and disseminated infection and develop immunity to reinfection. Normal rabbit serum (NRS) was obtained from 10 normal rabbits. Individual serum samples were stored at -80°C until ready for use. At the time of initial serum injection, equal amounts of individual IRS or NRS were pooled, heat-inactivated at 56°C for 30 minutes, and filter-sterilized. As determined by enzyme-linked immunosorbent assay (ELISA), the pooled IRS had anti-B burgdorferi and anti-OspA titers of 1:16000, an anti-OspC titer of 1:1000, an anti-DbpA titer of 1:2000, and an anti-DbpB titer of 1:1000. The pooled NRS had baseline antibody reactivity of <1:125 to B burgdorferi, OspC, DbpA, and DbpB and <1:250 to OspA. Both pooled sera were aliquoted and stored at -80°C until ready for use.

Passive Immunization and Challenge of Rabbits

Groups of 5 rabbits, each weighing approximately 2.3 kg, were injected intravenously (IV) at various time points via the marginal ear vein with 3 mL per kg body weight of either heat-inactivated, undiluted pooled IRS or NRS, or sterile PBS, pH 7.2. Sera or PBS given to rabbits before and after challenge (IRS “Before,” NRS “Before,” and PBS groups) were administered at -18, 0, +24, +48, +96, and +216 hours relative to challenge; sera given to rabbits only after challenge (IRS “After” and NRS “After” groups) were administered at +24, +48, +96, and +216 hours relative to challenge. As a control for B burgdorferi infection and EM development, one group of rabbits was given neither sera nor PBS (naive group).

Each of the rabbits was challenged ID at the 0 hour time point with 0.1 mL of the challenge inoculum at each of six sites for a total of 6 × 10⁶ B burgdorferi.

Skin Biopsy and Tissue Collection and Culture

Skin punch biopsies were obtained from all rabbits at the time of EM development in the control, naive rabbits (day 8). Rabbits were anesthetized by intramuscular injection with 45 mg Ketaset (Fort Dodge Laboratories, Fort Dodge, IA) and 8.8 mg Xylazine (Lloyd Laboratories, Shenandoah, IA) per kg body weight. A 4 to 5 mm sterile punch biopsy (Baker and Cummings, Lakewood, NJ) was taken adjacent to the inoculation site from the clipped back of each rabbit. Each biopsy specimen was immediately minced and cultured in 5 mL of BSK II medium containing 100 μg phosphomycin per mL and 50 μg rifampin per mL (Sigma, St. Louis, MO).

At 3 weeks post-challenge (pc), rabbits were bled from the central ear artery. Skin punch biopsies, popliteal lymph nodes, joint tissue surrounding the patella, and spinal cord were aseptically removed immediately after each rabbit was sacrificed by lethal IV injection of 100 mg sodium pentobarbital per kg body weight. Portions of each tissue were minced and cultured in BSK II medium containing 100 μg phosphomycin per mL and 50 μg rifampin per mL.

Erythema migrans skin biopsy cultures were incubated aerobically at 34°C for a period of 7 weeks while skin biopsies and viscera obtained at the time of death were
incubated under the same conditions for 15 weeks. The presence or absence of *B. burgdorferi* was determined periodically by darkfield microscopy. Cultures were considered negative when no spirochetes were observed during the above indicated observation periods.

**ELISA**

For determination of serum antibody levels against *B. burgdorferi*, OspA, OspC, DbpA, and DbpB, each of the rabbits was first prebled (basal specimen) from the central ear artery and then bled after administration of sera or PBS. Rabbits were bled at the 0 (Before groups only), +24, +96, and +216-hour time points in the same order that they received sera or PBS. The serum samples were stored at -80°C until assayed.

Flat-bottomed 96-well immunoassay plates (Immuno 4, Dynatech Laboratories, Inc., Chantilly, VA) were precoated overnight at room temperature with 100 µL containing 1 mg/mL of either sonicated whole *B. burgdorferi* B31, passage 3, recombinant OspA (kindly provided by SmithKline Beecham Pharmaceuticals, Rixensart, Belgium), recombinant OspC (kindly provided by Steven M. Callister and Steven Lovrich, Gunderson Lutheran Medical Center, La Crosse, WI), recombinant DbpA, or recombinant DbpB (tDbps kindly provided by Magnus Höök, Texas A&M University, Houston, TX) in PBS, pH 7.2. The plates were washed three times with PBS containing 0.05% Tween 20 (PBS/Tween) and blocked with BLOTTO (5% nonfat dry milk in PBS). After three washes, serum samples serially diluted two-fold in PBS, starting at 1:125 for anti-*B. burgdorferi*, OspC, DbpA, and DbpB ELISAs or 1:250 for anti-OspA ELISAs, were added to the wells and incubated at room temperature for 2 hours. Plates were washed three times and then incubated with donkey antiseraum against rabbit Ig conjugated to horseradish peroxidase (1:5000) (Amersham Corp., Arlington Heights, IL) at room temperature for 1 hour. The plates were then washed three times and incubated with 2, 2'-azinobis (3-ethyl benzthiazoline sulfonic acid) peroxidase substrate system (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) at room temperature in the dark for 30 minutes. The reaction was stopped with 1% sodium dodecyl sulfate (SDS) and the absorbance of each well was measured at 405 nm with an automated ELISA plate reader (Titertek Multiscan MCC/340; Flow Laboratories, Baar, Switzerland). Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.

**RESULTS**

**Passive Protection Against B. burgdorferi Challenge**

To determine the protective capability of infection-derived immune rabbit serum against experimental *B. burgdorferi* infection, naive rabbits were passively immunized and challenged. Rabbits administered IRS both before and after challenge failed to develop EM lesions at any site and all sites were shown to be culture-negative (Table 1). In contrast, animals receiving IRS only after challenge developed culture-positive EM lesions (Table 1). As expected, rabbits receiving NRS either before or after *B. burgdorferi* challenge, as well as rabbits receiving only PBS, had culture-positive EM lesions comparable to those of the naive controls. The time of appearance of the EM lesions also differed between groups of rabbits.
### Table 2. Serum antibody responses to B burgdorferi following passive immunization and challenge.

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>GMT* Serum anti- B burgdorferi Ig</th>
<th>Time Post-Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>0 Hrs.¹</td>
</tr>
<tr>
<td>IRS³ Before</td>
<td>125</td>
<td>3200±1095</td>
</tr>
<tr>
<td>IRS After</td>
<td>&lt;125</td>
<td>N/A¹</td>
</tr>
<tr>
<td>NRS After</td>
<td>150±56</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.

¹Serum samples obtained before challenge.
²N/A, serum samples not obtained.
³IRS anti-B burgdorferi titer - 1:16000

Control rabbits (PBS and naive groups) were the first to develop EM lesions (day 7 pc), followed by rabbits receiving NRS before challenge (NRS Before group; day 7-8 pc), and then by rabbits receiving IRS after challenge (NRS After group; day 9 pc). The duration and diameter of the lesions did not differ significantly between these groups of animals. Rabbits receiving IRS only after challenge (IRS After group), while developing lesions at all sites, did not develop EM lesions until day 13 or 14 p.c. and the duration of the lesions was shorter than the other control groups.

To determine whether passive immunization with infection-derived immune rabbit serum protected against skin and disseminated infection, all rabbits were sacrificed 3 weeks post-challenge and skin and disseminated sites of dissemination evaluated for evidence of B burgdorferi infection. As shown in Table 1, rabbits receiving IRS both before and after challenge (IRS before group) were completely protected against challenge as evidenced by the absence of infection in the skin and at disseminated sites including the popliteal lymph nodes, joint tissue, and spinal cord. In contrast, rabbits given IRS only after challenge (IRS after group), as well as all control groups, showed both skin and disseminated infection.

#### Serum anti-B burgdorferi Antibody Titers at Intervals After Passive Immunization

Serum samples from the IRS before, IRS after, and NRS after groups were obtained at the 0 (IRS Before group only), +24-, +48-, and +216-hour time points after administration of sera and at 3 weeks post-challenge for the determination of B burgdorferi-specific antibodies by ELISA. As shown in Table 2, the levels of serum anti-B burgdorferi Ig did not differ significantly between rabbits receiving IRS both before and after challenge and rabbits receiving IRS only after challenge. As expected, the anti-B burgdorferi antibody titer of rabbits receiving NRS at the earlier time points was comparable to that of the basal level measured for the NRS pool. At 3-weeks post-challenge, rabbits that received IRS and were free from local and disseminated infection (IRS Before group), had a five-fold lower B burgdorferi-specific antibody titer compared to the titer obtained at the 216 hours post-challenge time point. Rabbits in the IRS After group also showed a decrease in the anti-B burgdorferi antibody titer at 3 weeks post-challenge compared to the 216-hour time point; however, these animals showed both local and disseminated infection. By comparison, rabbits in the NRS After group had a 20-fold increase in anti-B burgdorferi Ig titers between the 216-hour and 3-week post-challenge time points consistent with active infection.

#### Comparison Between Status of Immunity and Anti-OspA, Anti-OspC, Anti-DbpA, and Anti-DbpB Antibody Titers

Several investigators have indicated that antibody against OspA, OspC, and DbpA in mouse immune serum may play a pivotal role in protection against challenge with B burgdorferi. To determine whether antibodies against these specific proteins play a similar role in protection against challenge in the rabbit model, serum antibody levels against OspA, OspC, DbpA, and also DbpB were determined by ELISA following passive immunization.

During the course of immune serum administration, the antibody titers against OspA in rabbits completely immune to challenge were found to be relatively low ranging from 1:800±274 to 1:2,400±894. In this same group, the anti-OspC titers of ≤1:125 were comparable to those obtained for basal sera (Table 3). Further, the anti-OspA and anti-OspC Ig titers did not differ significantly between rabbits from the IRS Before group and rabbits from the IRS After group despite the fact that only rabbits in the Before group were completely protected against challenge (Table 3).
Table 3. Comparison of immune status of passively immunized rabbits and anti-OspA and anti-OspC antibody titers.

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Rabbit Immune Status</th>
<th>GMT* Serum Anti-OspA Ig (Time Post-Challenge)</th>
<th>GMT* Serum Anti-OspC Ig (Time Post-Challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal</td>
<td>0 Hrs. 1</td>
</tr>
<tr>
<td>IRS5 Before</td>
<td>Immune</td>
<td>&lt;250</td>
<td>2000</td>
</tr>
<tr>
<td>IRS After</td>
<td>Non-immune</td>
<td>&lt;250</td>
<td>N/A 1</td>
</tr>
<tr>
<td>NRS After</td>
<td>Non-immune</td>
<td>&lt;250</td>
<td>N/A 1</td>
</tr>
</tbody>
</table>

*Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.

1Serum samples obtained before challenge.

IR5 anti-OspA titer - 1:16000
IR5 anti-OspC titer - 1:1000

Similarly, the serum antibody titers against DbpA and DbpB were relatively low in each of the passively immunized rabbits (Table 4). During the first 24-hour course of serum administration, no significant differences in the anti-DbpA and anti-DbpB titers were observed among the passively protected and unprotected groups of animals; the titers were comparable to those of the basal sera (<1:125) (Table 4). However, at the 3-week post-challenge time point, rabbits from the IRS After and NRS After groups developed significantly higher antibody levels against DbpA and DbpB as compared to the IRS Before group. These titers ranged from 1:1100±548–1:6400±2191. In contrast, rabbits from the passively protected IRS Before group had no significant increases in their anti-DbpA or anti-DbpB titers compared to their basal sera levels throughout the entire 3-week observation period.

**DISCUSSION**

In this study, we have shown that the high degree of infection-derived immunity that develops in the rabbit Lyme disease model can be transferred to naïve recipients by passive immunization with serum, indicating that antibody is a major contributor to this protective response. The ability of passively administered immune serum to protect against challenge with cultured homologous *B burgdorferi* has also been demonstrated in the murine model.21,23-28 The murine model is known to be a permissive host for *B burgdorferi* infection, which may relate, in part, to the inability of the murine immune response to clear the infection. By comparison, the immune response in the rabbit results in clearance of infection and complete immunity to reinfection, which may be caused by differences in antigen presentation.

Rabbits receiving immune serum were completely protected against development of EM, dermal infection, and visceral infection for at least 3 weeks after challenge. Complete protection against infection was achieved only when immune serum was administered before challenge (Table 1, IRS Before group). Once infection had been established, passively administered immune serum could not inhibit the establishment of infection and these animals eventually developed EM and dermal and visceral infection (Table 1, IRS After group). A similar observation has been reported for the murine and hamster models of passive immunity where immune serum given after challenge did not eliminate infection.21,28,29 However, EM appearance in rabbits given immune serum after challenge was significantly delayed as compared to control animals. In addition, these animals showed significantly lower anti-*B burgdorferi* antibody titers at 3 weeks post-challenge as compared to controls. Taken together, these observations suggest that immune serum given only after challenge, while not completely protective, reduced the number of virulent organisms. The reasons for the inability of immune serum to completely protect when given after challenge are not known, but may relate to what has been termed "host-adap-
Table 4. Comparison between the status of immunity of passively immunized rabbits and anti-DhpA and anti-DhpB antibody titers.

<table>
<thead>
<tr>
<th>Group (n=5)</th>
<th>Rabbit Immune Status</th>
<th>GMT* Serum Anti-DhpA Ig (Time Post-Challenge)</th>
<th>GMT* Serum Anti-DhpB Ig (Time Post-Challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Basal 0 Hrs.</td>
<td>24 Hrs.</td>
</tr>
<tr>
<td>IRS Before</td>
<td>Immune</td>
<td>&lt;125</td>
<td>250</td>
</tr>
<tr>
<td>IRS After</td>
<td>Non-immune</td>
<td>&lt;125</td>
<td>N/A</td>
</tr>
<tr>
<td>NRS After</td>
<td>Non-immune</td>
<td>&lt;125</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Antibody levels are expressed as geometric mean titer (GMT) for an optical density of 0.1 calculated from a plot of the mean responses ± standard deviations of 5 animals through 11 doubling dilutions. A significant antibody response was defined as a four-fold or greater rise in titer.

1Serum samples obtained before challenge.
2N/A, serum samples not obtained.
3IRS anti-DhpA titer: 1:2000
4IRS anti-DhpB titer: 1:1000

Organisms that have adapted to the host environment are known to upregulate some proteins and express de novo other proteins not found with either cultivated or tick adapted organisms. This property together with the known host-adapted downregulation of other proteins, including the major surface protein OspA, could conceivably make host-adapted organisms less susceptible to the protective effects of immune serum.

*burgdorferi*-specific ELISA titers were also consistent with the response to challenge of the passively immunized rabbits. Active infection in the rabbits receiving NRS showed a 20-fold increase in anti-*B burgdorferi* antibody titers between the 216-hour (9-day) and 3-week post-challenge time points. By comparison, rabbits immune to challenge (IRS Before group) had a five-fold lower anti-*B burgdorferi* antibody titer at 3 weeks post-challenge as compared to the titer at 216 hours post-challenge, which is consistent with the clearance of spirochetes. As mentioned above, rabbits receiving IRS after challenge (IRS After group), which had delays in EM appearance but were shown to be infected, did not show an increase in titer but rather had a 2-fold decrease in titer between these same time points, again suggesting that passive transfer of immune serum given after challenge may have reduced the number of virulent organisms.

Anti-OspA antibody titers in protected rabbits (Before group) were relatively low during the course of passive immunization and following challenge (≤1:2400±894). We have previously reported that rabbits immunized with purified OspA and exhibiting antibody levels of 1:40000 were not completely protected against *B burgdorferi* challenge. Barthold and colleagues 21,27 have also reported that active infection of mice induces a stronger protective humoral immune response than immunization with OspA, and that no correlation exists between the protective capability of immune serum and OspA antibody. Taken together, these findings support a conclusion that antibodies against OspA do not contribute to the protective response of passively transferred immune rabbit serum. The further observation that OspA antibody titers in infected rabbits from the IRS After group, were relatively low following challenge (≤1:2400±894) and did not increase comparable to the antibody titers against whole organisms, is consistent with the downregulation of OspA expression in rabbit host-adapted organisms similar to what has been observed in rodents.

Among passively immunized rabbits that were completely protected, titers of anti-OspC antibody at each time point following immunization and challenge were relatively low (≤1:125) and comparable to the normal sera titers (<1:125 to 1:475±335). Thus, the very low levels of OspC antibody detected suggest that OspC antibodies in immune rabbit serum used for passive transfer were not a major contributor to the protective immunity conferred. Alternatively, we cannot rule out at this time that this very low level of anti-OspC antibody was not a factor, although, several observations by other investigators showing that
immunization with OspC stimulates incomplete or no protection in mice using a homologous as well as heterologous challenge. The serum antibody titers against DpbA (peak titer: 1:300±112) and DpbB (peak titer: 275±205) among completely protected rabbits were also relatively low at the time of challenge (1:250 and 1:175±68, respectively) and comparable to the normal sera titers (<1:125). In addition, these titers did not differ significantly from the basal sera levels (<1:125) during the entire 3-week observation period. These data, together with the observations by other investigators that active immunization of mice with DpbA does not result in complete protection against challenge with cultured or host-adapted B burgdorferi, suggest that DpbA antibodies do not play a primary role in the protective immunity afforded by infection-derived immune rabbit serum.

In summary, we have demonstrated the ability of infection-derived immune rabbit serum to completely protect against homologous B burgdorferi challenge using cultured spirochetes. Our findings further suggest that antibodies present in infection-derived immune rabbit serum directed against target antigens other than OspA, and possibly OspC, DpbA, and DpbB are involved with this protection. The identification of antigens that are the targets for passive protection should provide molecules for new vaccine candidates.

ACKNOWLEDGMENTS

This work was supported by NIH grant AI-37312 to J.N. Miller and NIH grant AI-29733 to M.A. Lovett. We thank Dr. Ellen Shang for helpful suggestions to this study.

REFERENCES

25. Schable UE, Wallisch R, Kramer MD, et al. Immune sera to individual Borrelia burgdorferi isolates or recombinant OspA thereof protect SCID mice against infection with homologous strains but only partially or not at all against those of different OspA/OspB genotype. Vaccine 1993;11:1049-1054.
Laboratory and Clinical Findings in Tick-Borne Encephalitis—
Discrimination from Neuroborreliosis

R. Kaiser, PD

ABSTRACT

Laboratory parameters that might help to differentiate between neuroborreliosis (NB) and tick-borne encephalitis (TBE) are investigated. One hundred patients with TBE and 67 patients with acute NB were examined for the total cell count and protein content in the cerebrospinal fluid (CSF), the CSF/serum albumin ratio, and the intrathecal synthesis of IgG, IgM, and IgA. Pleocytosis and impairment of the blood-CSF-barrier were common findings in both diseases, whereas oligoclonal IgG and IgM bands in the CSF and intrathecal synthesis of IgM and IgG (P < 0.001), and of IgA (P = 0.010) as determined by quantitative measurements, were more common in patients with NB. Patients with NB had higher cell counts in the CSF (P = 0.001), a more severe impairment of the blood-CSF-barrier (P < 0.001), and the fraction of intrathecally produced immunoglobulins was higher (P < 0.001).

Regarding a broad range of individual parameters in patients with TBE and NB, no diagnostic conclusion can be drawn from the assessment of single laboratory parameters. The most supportive laboratory parameters for NB are the presence of oligoclonal IgM and IgG bands in the CSF and elevation of the IgG index (> 0.70). Tick-borne encephalitis however, is more likely when patients present with a biphasic course of illness, high grade fever, moderate pleocytosis with presence of neutrophilic leukocytes, and mild elevation of the total protein or CSF/serum albumin ratio.

Key words: neuroborreliosis, tick-borne encephalitis, TBEV

INTRODUCTION

In Europe, the most important tick transmitted diseases are Lyme borreliosis, caused by the spirochete Borrelia burgdorferi sensu lato, and tick-borne encephalitis (TBE) caused by TBE virus (TBEV), a member of the genus flaviviruses within the family Flaviviridae. Most areas in northern, central, and eastern Europe which are endemic for TBEV are also endemic for B. burgdorferi sensu lato, but the reverse is not always true.1 Tick-borne encephalitis virus infection occurs mostly as a febrile illness. The main clinical neurological syndromes associated with TBE are meningitis (40-50%), meningoencephalitis (40-50%), and meningoencephalomyelitis (10-15%).2-5 Lyme borreliosis is a multisystem disorder involving skin, joints, the heart, and frequently the central nervous system.6-7 Therefore, if subjects who live in areas endemic for TBEV fall ill with neurological symptoms, both diseases have to be considered, especially when a tick bite has been reported. In the majority of patients, TBE can be distinguished from neuroborreliosis (NB) on the basis of clinical symptoms. In individual patients, however, neuroborreliosis may occur as a febrile encephalitis or myelitis, mimicking the typical presentation of TBE. Both diseases can be diagnosed reliably by serological tests, but this may result in some delay, depending on local availability of resources. In this situation, other laboratory findings might be useful to support the suspected diagnosis. The aim of this prospective study was to investigate particular CSF parameters that might be characteristic for either disease, in order to support the suspected diagnosis.

MATERIAL AND METHODS

A total of 100 consecutive patients with TBE (67 males, 33 females, median age 47 years, range 16-82 years), and 67 patients with NB (41 males, 26 females, median age 40 years, range 19-80 years), admitted to the Neurological Clinic of the University of Freiburg between 1990-1997, were tested. All patients lived in an area endemic for TBEV and B burgdorferi sensu lato (Black Forest region in southern Germany).

Tick-borne encephalitis virus infections were con-

From the Department of Neurology, University of Freiburg, Freiburg, Federal Republic of Germany.

Address correspondence to R. Kaiser, PD, Department of Neurology, University of Freiburg, Breisacher Straße 64, D-79106 Freiburg, Federal Republic of Germany.
firmed by demonstration of TBEV-specific IgM and IgG antibodies in serum by routine serological screening tests (IMMUNOZYM FSME IgG, IgM, IMMUNO AG, Heidelberg, Federal Republic of Germany). Specimens were tested at the Department of Virology, Institute of Medical Microbiology, University of Freiburg. Samples were included in this study only when they showed an intrathecal synthesis of TBEV-specific IgG antibodies, IgM antibodies, or both at presentation or on a second analysis 4 weeks at the latest. Patients with TBE were not included in this study when they showed IgM or IgG antibodies to B. burgdorferi sensu lato in serum or CSF.

The diagnosis of NB was based on neurological symptoms, pleocytosis, and assessment of B. burgdorferi sensu lato specific IgM antibodies, IgG antibodies, or both in serum by a positive titer (IgM >1:24, IgG >1:32) in an indirect immunofluorescence assay. Specimens were tested at the Department of Immunology, Institute of Medical Microbiology, University of Freiburg. The diagnosis was confirmed by the demonstration of an intrathecal synthesis of B. burgdorferi sensu lato-specific IgG or IgM antibodies at presentation or at a second lumbar puncture after 4 weeks at the latest. None of the patients with neuroborreliosis showed IgM or IgG antibodies to TBEV in serum.

**Standard CSF Determinations**

Cells in the CSF were sedimented onto slides by centrifugation for 3 minutes at 800 rpm in a cytocentrifuge (Hettich Rotix/A, Tuttingen), air dried, and visualized by May-Grunwald and Giemsa stain. Albumin, IgG, IgM, and IgA were measured in CSF and the corresponding serum by laser nephelometry (Behring Nephelometer 100). With this, apparatus detection limit for IgG is 11 mg/L and for IgM and IgA 1 mg/L. The blood-CSF-barrier was evaluated by determination of the albumin CSF/serum ratio. Intrathecal synthesis of immunoglobulins (IgG, IgM, and IgA) was calculated by Reiber's formula. Analysis of oligoclonal IgG and IgM bands in the CSF and serum was performed as described previously.

**ELISA**

Intrathecal synthesis of agent-specific IgG and IgM antibodies was determined by ELISA as described previously. Antibodies to TBEV were examined using commercially available microtiter plates pre-coated with inactivated TBEV particles (IMMUNOZYM FSME-IgM, IgG, Immuno AG Germany, Heidelberg). Antibodies to B. burgdorferi sensu lato were determined using microtiter plates coated with 100 µL of B. burgdorferi sensu stricto strain GeHo (20 µg/mL) sonicate in phosphate buffered saline. As a reference, antibody-positive sera from 20 patients with either TBE or borreliosis were pooled, prediluted 4000-fold for IgG and 2000-fold for IgM, and then diluted two-fold with 6 serial dilution steps. Standard dilutions were chosen so that the corresponding absorbency values were between 0.10 and 1.80 A. Samples to be tested were diluted 1000- and 4000-fold (serum) and 50- and 200-fold (CSF). Specimens were incubated for 1 hour. After washing, 100 µL of peroxidase-conjugated goat antihuman IgG (1:5,000, Dianova, Hamburg, F. R. G.), or antihuman IgM (1:2,000) was added. After 1 hour at room temperature the plates were washed and 100 µL of o-phenylenediamine (OPD) in citrate buffer (pH 5) containing 0.04% H2O2 was added to each well. The enzymatic reaction was stopped after 15 minutes by the addition of 30 µL of 2.5 N H2SO4. The optical densities (OD) at 492 nm were measured with a Dynatec MR400 reader. ODs, which were 3 SD above the mean of 20 negative controls, were considered to have a positive antibody response to B. burgdorferi sensu lato or TBEV. Samples were tested in duplicate and the mean value was calculated. Negative controls were included on every plate.

The standard values (reference sample) were evaluated in a log/log diagram (OD/relative concentrations). The greatest measurable standard concentration (OD 1.8) was defined to be 1,000 arbitrary concentration units. Final concentrations of antibodies raised against B. burgdorferi sensu lato or TBEV in CSF and serum were calculated by multiplication of arbitrary concentration units with the dilution factor. Specific antibody synthesis in the CNS was calculated by the Antibody Index (AI), which is defined as the ratio between the CSF/serum quotient for specific antibodies and the quotient of total IgG or IgM concentrations in CSF and serum. Any AI >1.5 was considered indicative of a local antibody response to B. burgdorferi sensu lato or TBEV.

**Statistical Methods**

The individual parameters in patients with moderate and severe disease were tested for significance by the Mann-Whitney U test, using SPSS/PC+ software. The frequency of positive results in both groups was examined by χ²-test (Fisher's exact test). The significance level considered was 1% (P < 0.01).

**RESULTS**

**Clinical and Serological Findings**

Thirty-four patients with TBE suffered from isolated meningitis, 46 from meningoencephalitis, and 20 patients from meningoencephalomyelitis. A prodromal stage with flu-like symptoms and low grade fever (<39°C) was present in 68 patients (68%). In all patients, TBE high grade fever (>39°C) and severe headache were the first symptoms causing hospitalization. Infections with the TBEV
<table>
<thead>
<tr>
<th>Parameter</th>
<th>TBE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=100)</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
</tr>
<tr>
<td>TBEV/Bb-specific IgM antibodies in serum</td>
<td>97</td>
</tr>
<tr>
<td>TBEV/Bb-specific IgG antibodies in serum</td>
<td>79</td>
</tr>
<tr>
<td>Intrathecal synthesis of TBEV/Bb-specific IgM</td>
<td>61</td>
</tr>
<tr>
<td>Intrathecal synthesis of TBEV/Bb-specific IgG</td>
<td>71</td>
</tr>
<tr>
<td>Intrathecal synthesis of TBEV/Bb-specific IgG/M</td>
<td>84</td>
</tr>
</tbody>
</table>

were acquired between March and October; 60 patients (60%) remembered a tick bite one to three weeks before the onset of clinical symptoms.

Fifty-nine patients (88%) with NB suffered from meningoradiculitis (Bannwarth’s syndrome), five (7%) from meningoencephalitis, and three (5%) from meningomyeloradiculitis. Thirty-one patients (46%) recalled a tick bite and 25 patients (37%) recalled a preceding erythema migrans. The frequency of agent-specific IgM and IgG antibodies in patients with TBE and neuroborreliosis on admission to hospital is presented in Table 1. All patients were positive for either IgM or IgG antibodies in serum.

CSF Findings on Admission to Hospital

The frequency of pathological findings in the CSF of patients with TBE and NB is shown in Table 2. The evaluation was done individually for each parameter and for combinations of at least two different parameters. The greatest differences between patients with TBE and NB were found with oligoclonal IgM (Δ 82%) and IgG (Δ 61%) bands in the CSF, and for oligoclonal IgG bands and an increased albumin CSF/serum ratio (Δ 54%) or an intrathecal synthesis of IgM as calculated by Reiber’s formula (Δ 54%). Of the parameters that can be determined rapidly by laser nephelometry, the IgG-index was most useful. Elevation of the IgG-index over 0.70 was more frequent in patients with NB than with TBE. The mean and standard deviations as well as the median and range of the routine CSF parameters are shown in Tables 3A and 3B. Pleocytosis of CSF was a general finding in all patients with TBE and NB. In patients with TBE, segmented granulocytes (60-70%) predominated over lymphocytes (30-40%) in about 50% of specimens, while in patients with NB mononuclear cells consisted mainly (>90%) of lymphocytes.

DISCUSSION

The focus of this study was to compare clinical and laboratory findings in patients with TBE and neuroborreliosis. None of the patients presented with an infection with both agents. In an epidemiological study in southern Germany serological evidence of previous infection with both agents was found in only 6 of 393 individuals (1.5%), while TBEV-specific antibodies were demonstrated in 34 (9.4%) and B burgdorferi sensu lato-specific antibodies in 53 (13.5%) inhabitants.13

When reliable serodiagnosis is not rapidly available, the diseases have to be distinguished on the basis of clinical symptoms and other laboratory findings. Incubation periods in TBE vary between one and three weeks (exceptionally four weeks), but are longer in acute neuroborreliosis, between three weeks and several months.14,15 As a rule, TBE presents as high grade fever, severe headache, a more or less intense depression of consciousness, and facultative neurological symptoms. These findings, however, are rarer in patients with neuroborreliosis, who usually present with pain and pareses of the extremities and cranial nerves (predominantly the facial nerve).

Both diseases can be differentiated by serological tests without problems. On admission to hospital, specific IgM antibodies in serum were present in 97% of patients with TBE, but in only about 50% of patients with neuroborreliosis, a finding that has been reported by a number of other authors.16-22 In TBE, specific IgM antibodies in serum are the main diagnostic criterion used to confirm the suspected diagnosis. In a small number of cases, (eg, in patients with passive immunization), the IgM response may be delayed or even absent. In these latter patients, ongoing viral infection may sometimes be identified by determination of the avidity of IgG antibodies directed against TBEV.23 The rare finding of specific IgM antibodies in NB might be explained by the longer period between infection and occurrence of neurological symptoms in most of these patients. On the other hand, in individual patients with borreliosis-specific IgM antibodies have been detected—by immunoblot—for up to five years,24 while in TBE-specific IgM-antibodies, may persist for up to nine months after the acute illness.19

Sometimes investigation of the CSF, done immediately after lumbar puncture, may yield useful information earlier than receipt of serological findings. Pleocytosis in the CSF was a common finding in both diseases, but the mean cell count in NB was significantly higher than in TBE. The data from patients with TBE are in line with data in previous reports from Gunther et al25 who
Table 2. Frequency of pathological findings in tick-borne encephalitis (TBE) and acute neuroborreliosis (NB). Noticeably higher frequencies of pathological findings in patients with TBE are printed in bold.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TBE (N=100) (%)</th>
<th>NB (N=67) (%)</th>
<th>Differences in Frequency (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased total protein in the CSF</td>
<td>84</td>
<td>85</td>
<td>1</td>
<td>0.500</td>
</tr>
<tr>
<td>Increased Albumin CSF/serum ratio</td>
<td>80</td>
<td>90</td>
<td>10</td>
<td>0.200</td>
</tr>
<tr>
<td>Oligoclonal IgG bands</td>
<td>27</td>
<td>88</td>
<td>61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oligoclonal IgM bands</td>
<td>0</td>
<td>82</td>
<td>82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG-index</td>
<td>19</td>
<td>66</td>
<td>47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG-synthesis</td>
<td>24</td>
<td>67</td>
<td>43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgM-synthesis</td>
<td>49</td>
<td>82</td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgA-synthesis</td>
<td>14</td>
<td>33</td>
<td>19</td>
<td>0.010</td>
</tr>
<tr>
<td>Increased Albumin CSF/serum ratio and increased IgG-index</td>
<td>16</td>
<td>61</td>
<td>45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increased Albumin CSF/serum ratio and increased IgG-synthesis</td>
<td>20</td>
<td>58</td>
<td>38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increased Albumin CSF/serum ratio</td>
<td>43</td>
<td>70</td>
<td>27</td>
<td>0.001</td>
</tr>
<tr>
<td>and increased IgM-synthesis</td>
<td>14</td>
<td>31</td>
<td>17</td>
<td>0.020</td>
</tr>
<tr>
<td>Increased Albumin CSF/serum ratio and increased IgA-synthesis</td>
<td>21</td>
<td>75</td>
<td>54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oligoclonal IgG bands and increased albumin CSF/serum ratio</td>
<td>19</td>
<td>73</td>
<td>54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oligoclonal IgG bands and intrathecal synthesis of IgM</td>
<td>9</td>
<td>27</td>
<td>18</td>
<td>0.003</td>
</tr>
<tr>
<td>Oligoclonal IgG bands and intrathecal synthesis of IgA</td>
<td>21</td>
<td>60</td>
<td>39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intrathecal synthesis of IgG and IgM</td>
<td>10</td>
<td>26</td>
<td>16</td>
<td>0.010</td>
</tr>
<tr>
<td>Intrathecal synthesis of IgG and IgA</td>
<td>11</td>
<td>29</td>
<td>18</td>
<td>0.005</td>
</tr>
<tr>
<td>Intrathecal synthesis of IgG, IgM, and IgA</td>
<td>35</td>
<td>65</td>
<td>30</td>
<td>0.003</td>
</tr>
</tbody>
</table>

described a mean CSF cell count of 90/μL. In other studies, the mean cell count in patients with TBE varied between 200 and 300/μL.3,26,27 In NB, the mean cell count has been reported to range from 110 to 370/μL, but in individual patients this value varies between 6 and 1830/μL.28-35 In view of the high variability and the overlap of the mean cell counts in TBE and NB, this criterion is invalid for the differential diagnosis of both diseases. Differentiation of cells in the CSF by phase microscopy may be more useful. In most patients, NB lymphocytes predominate over neutrophilic leukocytes, which are present only occasionally. The most obvious finding, however, is the presence of activated B-cells and plasma-cells in the CSF.18,36 Such cells are usually absent in the CSF of patients with TBE, where about 50% of patients show a predominance of neutrophilic leukocytes, which may persist for several days.37,38 However, when small lymphocytes are the predominant cell type in the CSF, no conclusion can be drawn with respect to TBE or NB. Impairment of the blood-CSF-barrier is a frequent finding in both diseases. The most accurate method to assess a dysfunction of this barrier is measurement of the albumin CSF/serum ratio.9 As some authors still prefer measurement of the total protein content in the CSF instead of the albumin CSF/serum ratio, both measurements were made in this study. The finding of moderate impairment of the blood-CSF-barrier in patients with TBE but more severe impairment in patients with NB is in line with previous reports: Gunther et al.39 investigated 85 patients with TBE and found an average albumin CSF/serum ratio of 9-10×10⁻³. In two further studies (N=126 patients), the average total protein ranged from 840 to 1050 mg/L.30,41 Patients with neuroborreliosis generally show a more severe impairment of the blood-CSF-barrier. Henrikkson et al.42 described five patients with NB who presented with a median albumin CSF/serum ratio of 16.3×10⁻³ (range 14.6-30.2). In another study, the albumin CSF/serum ratio ranged between 7.8 and 82×10⁻³; no information was
given about the median or mean values.53 In studies where the mean total protein content in the CSF instead of the albumin CSF/serum ratio was determined, values ranged from 1200-1900mg/L.28,30,44,45 Even though these results are largely confirmed by the findings in this study and comparison of study groups speaks for more severe damage of the blood-CSF-barrier in patients with NB, in individual subjects the albumin CSF/serum ratio is of limited use. Pathological findings in the range from 6.7 to 47×10^{-3} can result from infection with either of these tick transmitted agents.

The most obvious laboratory criterion for differentiation of NB from TBE was the demonstration of oligoclonal IgM bands in the CSF. The presence of these bands is highly indicative of neuroborreliosis, when a lymphoma of the CNS can be excluded.10 While in patients with NB the presence of these bands was correlated with increased fractions of locally produced IgM—as calculated by Reiher’s formula—no such correlation could be demonstrated in patients with TBE. In the latter patients intrathecal synthesis of IgM was proven by quantitative measurements but not by isoelectric focusing. The failure to detect oligoclonal IgM bands in patients with TBE probably indicates a more polyclonal pattern of locally produced IgM in these patients. In NB, oligoclonal IgM bands might result from a more chronic stimulation of B-cells by certain proteins (p31) of B burgdorferi sensu lato.46 Analysis of oligoclonal IgM bands is difficult and not standardized in most laboratories. Therefore, quantitative measurements will be preferred by most investigators. Patients with NB showed not only a higher frequency but also higher fractions of locally produced IgM. In individual patients with TBE and NB, however, there was a considerable overlap of percentage values, decreasing the diagnostic significance of this observation.

Oligoclonal IgG bands in the CSF were present more frequently in patients with NB than with TBE. This finding was confirmed at least partially by the results of quantitative measurements of intrathecally produced IgG. Unexpectedly, combined evaluation with other parameters did not increase the differentiating ability of oligoclonal IgG bands. The magnitude of locally produced IgG fractions, as calculated by Reiher’s formula and the IgG index, was higher in patients with NB than with TBE. In individual subjects, however, there was a considerable overlap of values, decreasing the diagnostic significance of this finding.

Determination of oligoclonal bands is time-consuming and more expensive than quantitative measurements of immunoglobulins. With respect to this handicap, determination of the IgG index is the most rapid and inexpensive way to support the clinical assumption of TBE or NB. Elevation of the IgG index is more common in neuroborreliosis than in TBE.

Tick-borne encephalitis is likely in patients who fulfill the following criteria: recall of a tick bite in an area endemic for TBEV, biphasic course of disease, moderate pleocytosis with presence of neuropathic leukocytes in the CSF, mild elevation of total protein or Q-Alb, and intrathecal synthesis of low amounts of IgM. Suspected diagnosis is to be confirmed by the demonstration of TBE-specific IgM and IgG antibodies in serum. Demonstration of intrathecal synthesis of specific antibodies is useful if a previous infection has to be verified at a later time, but this criterion is not required to confirm the diagnosis during acute illness.

**ACKNOWLEDGMENT**

This work was supported by the Bundesministerium für Bildung und Forschung (01KI 9504/9). I’d like to thank P. Völker for her expert and skilful technical assistance and Professor Batsford for help in editing the manuscript.
REFERENCES


Detection of Borrelia DNA in Circulating Monocytes as Evidence of Persistent Lyme Disease

Ping Wang, MS; Ronald Gartenhaus, MD; Sunil K. Sood, MD; James DeVoti, PhD; Carol Singer, MD; Gary Dorante, BS; and Eileen Hilton, MD

ABSTRACT

We report the detection of Borrelia burgdorferi DNA in circulating monocytes in a 31-year-old female who presented with a flu-like syndrome followed by neurological abnormalities after a trip to Southampton, Long Island, New York. ELISA and Western blot were negative. Lymphocyte proliferation assay to Borrelia burgdorferi was positive. Borrelia burgdorferi DNA was detected in circulating monocytes using a nested polymerase chain reaction (PCR). Treatment with parenteral ceftriaxone resulted in clinical improvement and repeat PCR on monocytes was negative. The use of detecting DNA by PCR from circulating monocytes may be useful in evaluating seronegative patients with a high suspicion of Lyme disease.

Key words: Borrelia burgdorferi, PCR, seronegative Lyme disease

INTRODUCTION

The diagnosis of Lyme disease requires positive serologic evidence of Borrelia burgdorferi infection in the appropriate clinical setting. Seronegative Lyme disease has been described and frequently presents a diagnostic dilemma for the clinician. Alternative diagnostic tests include lymphocyte proliferation and antigen detection assays. These methods have several drawbacks, including lack of standardization, as well as poor sensitivity and specificity. In this report, we describe a patient with neurological symptoms suggestive of multiple sclerosis who had evidence of B burgdorferi present in her circulating monocytes.

CASE REPORT

In 1993, immediately following a camping trip to Southampton, Long Island, a 31-year-old female resident of Nassau County, Long Island developed a mononucleosis-like syndrome with severe sore throat, neck tenderness, and profound fatigue. The sore throat resolved, but fatigue persisted. She began to have bilateral headaches, weakness on her left side, and difficulty walking. She also noted difficulty with concentration and word finding. A magnetic resonance imaging study done in September 1995 showed punctate linear areas of increased signal in the white matter, which were atypical for multiple sclerosis. She subsequently developed knee and ankle joint pain without swelling. She was diagnosed with depression in July 1994. A spinal tap in June 1996 revealed 7 white blood cells (WBC)/mm³ (all mononuclear), protein of 53 mg/mL, glucose of 58 mg/mL, and 3 oligoclonal bands. Cerebral spinal fluid VDRL was negative. On physical examination in August 1996, she was afebrile and well appearing. Neurological examination revealed a left pronator drift and left upper extremity weakness. She was unable to perform tandem walking and tended to drag her left foot. Finger-to-nose testing on the left side was abnormal. The remainder of the examination was within normal limits, including a rheumatological examination. Laboratory evaluation at this time revealed an antinuclear antibodies (ANA) titer of 1:80 (speckled) and an erythrocyte sedimentation rate of 32 mm/hr. VDRL was negative. Serological testing for Lyme disease showed negative ELISA and immunoblot assays in serum and cerebrospinal fluid. A lymphocyte
proliferation assay to *B. burgdorferi* performed by specialty Laboratories (Santa Monica, CA) was markedly positive with a Lyme stimulated counts per minute (CPM) value of 18,741 and a Lyme stimulation index (SI) of 40.4 (stimulated CPM > 10,000 and Lyme SI > 10 considered positive). A sonicated extraction of whole cell was used in the assay.

The patient received parenteral ceftriaxone for 6 weeks with improvement in her gait, left-sided strength, and resolution of the pronator drift. She also reported disappearance of all joint pain. A lumbar puncture at the end of treatment showed 1 WBC/mm³, protein of 43 mg/mL, and glucose of 58 mg/mL with 7 oligoclonal bands detected.

**MATERIALS AND METHODS**

Monocytes were isolated and tested for *B. burgdorferi* DNA as follows: 25 mL of whole blood from the patient, from 2 healthy donors, 5 osteoarthritic patients, and from a patient with multiple sclerosis were collected separately in heparinized tubes. The technologist was blinded to all clinical information. Whole blood was centrifuged over Ficoll-Hypaque medium (Organon Teknika, Durham, NC). A 5 mL suspension of the mononuclear cell layer was cultured in 25 cm² flasks. There were 200 µL of cell suspensions added to each chamber slide for 48-72 hours (RPMI 1640 medium with 10% fetal bovine serum, 5% CO₂ incubator at 37°C). Nonadherent cells (lymphocytes) and adherent cells (monocytes) were separated and washed.⁴ Cells maintained in flasks were subjected to DNA isolation, and cells on chamber slides were stained by immunohistochemistry to identify cell populations. Slides were washed 3 times with PBS, then air-dried and fixed in 100% acetone for 3 minutes. The staining procedure was performed per manufacturer’s instructions (Boehringer Mannheim Corporation, Indianapolis, IN) using mouse antihuman monocytes/CD 11b antibody. The majority (>90%) of adherent cells were confirmed to be monocytes (positive staining with mouse antihuman monocyte antibody), whereas <100% cells in the nonadherent fraction stained positive.

DNA was purified from each cell fraction with the Invitrogen Easy-DNA Kit (Invitrogen, San Diego, CA) per manufacturer’s instructions. A nested PCR assay designed to optimize detection of different OspA genotypes was modified from Moter et al.⁵ Briefly, external primers prZS7/3 1-1 and OspA-5 amplify a 662 base-pair (bp) fragment of the OspA gene, and internal primers OspA-6 and OspA-8 amplify a 392 bp fragment of the first product. The first PCR reaction mixture contained 30 µg total DNA, 1 × PCR buffer, 1.5 mmol/L MgCl₂, 200 µmol/L dNTP, 0.25 µmol/L of each external primer and 1.5 U of Taq DNA polymerase (Perkin Elmer, Branchburg, NJ). The total volume was 50 µL. PCR amplification reaction was performed in a DNA thermal cycler (Perkin Elmer) under the following conditions: Denaturation at 94°C for 90 seconds, primer annealing at 45°C for 120 seconds and extension at 72°C for 120 seconds, for 30 cycles. After the first reaction, 5 µL of the reaction mixture was added to a new PCR mixture containing 1 × PCR buffer, 1.5 mmol/L MgCl₂, 200 µmol/L dNTP, 0.25 µmol/L of each internal primer and 1.5 U of Taq DNA polymerase. Amplification conditions were changed to 35 cycles and annealing temperature 55°C. PCR products were analyzed on a 1.5% agarose gel. Ten fg of *B. burgdorferi* B31 DNA served as a positive control. PCR amplification for *B. burgdorferi* DNA has been used in our laboratory for several years without an incident of contamination. Four laboratories are used in a one-way-flow scheme so as to avoid contamination via re-amplification of amplicons. They included a dedicated room with dead-air hood for PCR set-up, room with thermal cycler, room with dedicated hood for second reaction set-up nested PCR, and a *dirty* room for analysis of products of amplification by ethidium bromide gel electrophoresis and Southern blotting. The laboratories used are in two different interconnected buildings, which allows us to completely separate the clean set-up and dirty areas for PCR.

To ensure that the DNA we isolated was amplifiable, 30 ng of cellular DNA was used to amplify exon 17 of the human amyloid precursor protein (APP) gene (primers, Research Genetics, Inc., Huntsville, AL).⁶ Primers that amplify a region of the human papilloma virus (HPV 11) genome and HPV DNA were kindly donated by Dr. Bettie Steinberg, LJI Department of Otolaryngology, to serve as a control for nonspecific amplification and were negative. PCR amplification conditions were 35 cycles, denaturation at 94°C for 60 seconds, annealing at 55°C for 120 seconds, and extension at 72°C for 120 seconds. As an additional control for the possibility of nonspecific amplification of unrelated bacterial DNA, a seminested PCR assay for amplification of *H. influenzae*, *N. meningitidis* and 16s eubacterial DNA was performed on the extracted DNA and was negative (data not shown).⁷

The gel was Southern blotted onto GeneScreen Plus nylon membrane (NEN Research Products, DuPont) with the use of standard conditions, then hybridized with a 25-bp probe (OspA-319, Nocton et al.)⁸ that was end labeled with fluorescein-11-dUTP (Amersham Life Science, Arlington Heights, IL). Chemiluminescent detection was performed with the ECL 3-oligolabelling and detection system (Amersham).
RESULTS

The PCR product of the APP gene was present on each sample except the human papilloma virus and B burgdorferi samples (see Figure). The patient’s pretreatment sample showed a PCR product corresponding to the intended OspA target. The healthy donor controls, arthritis controls, (data not shown) and the multiple sclerosis controls were negative. Specificity of the product was confirmed by Southern blotting. The OspA product disappeared following treatment.

DISCUSSION

We present a patient with a presumptive diagnosis of multiple sclerosis whom, three years after the onset of symptoms, had evidence of B burgdorferi DNA in her circulating monocytes. Since there was a clinical response to antibiotic treatment for Lyme disease, which was accompanied by disappearance of B burgdorferi DNA, it is likely that the patient’s signs and symptoms were because of, at least in part, chronic, seronegative B burgdorferi infection. Given the presence of oligoclonal bands in the CSF, it is also possible that the patient had concomitant multiple sclerosis and neuroborreliosis.

Seronegative Lyme disease has been described and been attributed in some cases to inadequate antibiotic treatment early in the infection. While the incidence of seronegative Lyme disease is unclear, several patients in endemic areas present with arthritic and neurological signs and symptoms but lack serologic evidence of B burgdorferi infection, and therefore do not fulfill the CDC criteria for a diagnosis of Lyme disease. Certainly, the possibility exists that some patients have latent, chronic synovial or CNS infection that cannot be detected by commonly available tests. Past reports have relied on lymphocyte proliferation assays for diagnosis, but critics have cited poor reproducibility, low specificity, and high cost. Recently, Morisset et al reported that B burgdorferi DNA can be detected in serum using a nested PCR method in seronegative patients. A European patient with myositis, negative serologic, and lymphocyte stimulation tests for Lyme disease, but PCR evidence of B burgdorferi in his peripheral blood mononuclear cells was described. The cell fraction was not specified, and the use of control specimens was not reported. Their patient had an atypical clinical picture but demonstrated a response to antibiotic therapy.

To the best of our knowledge, the present report is the first to demonstrate the presence of OspA gene segments from human monocytes. Monocytes including macrophages are highly active phagocytic cells that are present in both blood and tissues. Because antigens cannot directly activate lymphocytes, antigen-presenting cells, including macrophages, play a crucial role in the processing and presentation of antigens. The presence of B burgdorferi DNA in monocytes and the role of monocytes in killing have been described in vitro and in animal studies. It is possible that the monocyte can serve as a haven for the spirochete. The life span of circulating monocytes is approximately 2 to 5 days prior to their entry into tissues, where they may persist for long periods. It has been shown that B burgdorferi is ingested rapidly by mouse monocytes and degraded in lysosomes. There may be unknown host factors that can abort degradation, such as seen in HIV infection, where the virus may survive or proliferate in cells that are meant to eliminate it.

A variety of physiologic abnormalities, including those of apoptosis, glucocorticoid mediated monocyte function, and regulation of nitric oxide synthetase activity could cause monocytes to fail to present antigen to T-lymphocytes. Mouse monocytes can ingest pathogens without requiring prior opsonization with circulating antibody. Thus, the appearance of an antibody response may be delayed or abrogated in some patients. Perhaps similar mechanisms are responsible for some cases of seronegativity.

We cannot exclude the possibility that the B burgdorferi DNA represented past infection and was unrelated to the patient’s current symptoms. A beneficial response to placebo has been described in chronic fatigue syndrome patients with no documented infectious illness. The disappearance of the PCR product, though, is suggestive that treatment resulted in clearance of the organism.
This assay may be helpful in cases where Lyme disease is high on the differential but serology is negative. The presence of DNA does not automatically imply active infection. Nevertheless, it may be one factor in the decision to administer a trial of antibiotics in a likely clinical setting after other diagnoses have been excluded. In addition, it may be useful to follow the efficacy of antibiotic treatment with the realization that the presence of DNA does not necessarily mean active infection.

ACKNOWLEDGMENT

Supported by grants from the Helen & Irvine Schneider Family and LJI Faculty Research.

REFERENCES


13. Modolell M, Schable UE, Rittig M, Simon MM. Killing of Borrelia burgdorferi by macrophages is dependent on oxygen radicals and nitric oxide and can be enhanced by antibodies to outer surface proteins of the spirochete. Immunology Lett 1994;40:139-146.


Erythema Migrans—My Point of View

Edwin J. Masters, MD

There is an old saying that says “Wherever I take my eyes, I see things from my point of view.” Here is my point of view as a primary care physician in a CDC designated nonendemic area for Lyme disease related erythema migrans (EM).

I have been studying erythema migrans in the lower midwest since 1988. On one occasion I saw three EMs in one day, but never four—that is until May 26, 2000. Four cases are presented, all evaluated in a single day. There were 3 other EMs at our clinic earlier in that week. None had recent tick exposure in Lyme disease endemic areas. Over the past dozen years, I have evaluated between 20 and 35 EMs per year. Lyme disease in the lower Midwest and South is still controversial and most physicians do not report it.

Case #1 T.L.

A 42-year-old female removed a nymphal-sized tick from the left side of her abdomen 2 weeks previously. The rash started 1 week ago and has enlarged to its current size of 7 × 12 cm. She averages 3 tick bites per year and has never had such a reaction.

Case #2 D.E.

A 48-year-old female with a large tick exposure from gardening had an enlarging annular rash with a visible punctum. She has had no prior similar appearing rash. The annular erythema with central clearing was 7 cm in diameter. Additional complaints included dizziness, fatigue, and mild sore throat for 2 days.

Case #3 T.D.

A 43-year-old male removed an adult tick from his back 10 days previously at the site of the current rash, which was noticed the day before. He never previously had a rash following a tick bite. The rash was 6 × 9 cm with central clearing.

From Regional Primary Care, Inc., Cape Girardeau, Missouri.
Address correspondence to Edwin J. Masters, MD, Regional Primary Care, Inc., #69 Doctors’ Park, Cape Girardeau, MO 63703.
Case #4 J.L.

A 54-year-old male removed two imbedded nymph ticks on May 15 and May 18, which were obtained while walking near his pond. Only the tick bite in the groin area resulted in a spreading erythematous rash. The punctum was still visible. The 8×8 cm rash was partially obscured in the photo because of the hair. Previously, he never had a rash following a tick bite. Additional complaints included fatigue and myalgias for 4 days.

REFERENCES


Psychiatric Symptomatology Associated with Presumptive Lyme Disease: Clinical Evidence

Héctor R. Battaglia, MD*; Guido Alvarez, MD*; Augusto Mercau, MD*; Marcelo Fay, MD*; and Martín Campodónico*

ABSTRACT

In 43 patients with atypical depression, 7 experienced panic attacks and all considered hypochondriacal patients, were selected from the psychiatric office for having associated multisystemic polssymptomatology concordant to that of Lyme disease. The patients selected had lived or frequently visited areas at risk for zoonosis, been bitten by ticks, had a history of skin injuries or dermatosis compatible with erythema migran (EM), and had positive IgG and IgM serology for Borrelia burgdorferi. Combination therapy with specific antibiotic treatment for Lyme disease and specific psychotropics was indicated. Psychopharmacological therapy included a first 90-day period with monoaminoxidasa inhibitors followed by later periods with state-of-the-art tricyclic and tetracyclic antidepressants and serotonin-specific reuptake inhibitors.

Psychopathologies, as well as multisystemic polssymptomatology, diminished and even disappeared after the combination treatment. We conclude that an infectious disease whose closest definition could be Lyme disease produced the psychiatric symptoms described in this manuscript. Isolation of the causative agent remains to be done.

Key words: atypical depression, panic attack, hypochondriasis, multisystemic polssymptomatology, Lyme disease

INTRODUCTION

This study stems from clinical observations of patients assisted by a group of medical professionals concerned about the achievement of diagnosis certainty. These patients presented with atypical depression, panic attacks, and hypochondriasis as a common pattern of psychiatric pathology that was associated with wide, varied, and alternating clinical multisystemic polssymptomatology.

Atypical depression is described as a not otherwise specified mood disorder. It must meet certain precise criteria such as mood reactivity, significant weight gain or appetite increase, hypersonmia, depressed mood, and a long lasting pattern of sensitivity towards interpersonal rejection. It must not meet the diagnostic criteria for melancholic or catatonic symptoms.

Panic attack is defined as a crisis of anguish expressed as a temporary and isolated discrete period of intense fear or discomfort. It is accompanied by diverse physical symptoms that develop abruptly and reach a peak within 10 minutes.

Hypochondriasis is the exaggerated preoccupation and fear of the conviction of having a serious disease based on the personal interpretation of physical signs or sensations.

Professionals from medical specialities, including infectious diseases, previously assisted their patients but no final diagnosis had been reached. Multisystemic polssymptomatology has not been interpreted as emerging from only one disease in spite of multiple, complex, and costly complementary studies.

The therapies prescribed were inadequate, erroneous, and subsequently discontinued, given for an insufficient time period, and under symptomatic criteria. Patients had lost confidence in their doctors, medicine, and themselves and believed they had an incurable disease. None of the therapies prescribed to this group of patients from the various medical disciplines were successful.

Facing this clinical landscape and with the purpose of reaching a precise diagnosis, the search for a disease that included such multisystemic polssymptomatology was started, thereby bringing forth the idea of Lyme disease, a

From *Private practice and †Departamento de Biología Molecular CIBIC, Universidad Nacional de Rosario, Argentina.
Address correspondence to Héctor Battaglia, MD, Mat 3530 Medico Psiquiatra, Zeballos 1772, S2000CMP Rosario, Argentina.
disease not considered in this area because neither the etiological agent had been classified nor the vector identified.

Lyme disease, or Lyme borreliosis, is a zoonosis caused by the spirochete *Borrelia burgdorferi* that presents a multisystemic pathology. It is transmitted by an arthropod vector and has a worldwide geographic distribution.

Because Lyme disease has not been considered in atypical depression and other psychiatric disorders, the disease has not been broadly investigated in such syndromes. Patients in this study were in a nonacute stage of the suspected disease, carrying clinical pictures masked by antibiotic and corticoid therapies that complicated diagnosis and affected prognosis.

Clinical evidence and therapeutic responses highly compatible with Lyme disease point to the presence of Lyme disease in Argentina. Considered "the new great imitator" because of its multisystemic clinical presentations, this disease is found in its chronic stage because acute symptoms are attributed to other diseases.

Our clinical experience in psychiatric presentations of atypical depression, panic attack, and hypochondriasis presumptively associated with Lyme disease is hereby submitted.

**MATERIALS AND METHODS**

Forty-three patients diagnosed with atypical depression were selected from the psychiatric office, 7 of whom had experienced panic attacks. All were considered hypochondriacal patients. The group shared two characteristics: psychological and pharmacological. Psychologically, the patients demonstrated a positive rapport and satisfaction with the psychotherapeutical approach. Pharmacologically, the patients had poor or nonexistent response either to tricyclic and tetracyclic anti-depressants (TADs) or to serotonin-specific reuptake inhibitors (SSRIs), and positive reception of monoamine-oxidase inhibitors (MAOIs).

Selection of patients included the following criteria: psychiatric pathology associated to multisystemic poly-pathology variable for each individual case; permanent or frequent presence in areas at risk for zoonosis; tick bites; history of described skin injuries or dermatosis compatible with presumptive erythema migrans; and positive serology on IgG and IgM for *Borrelia burgdorferi*.

A control group was not presupposed. This study is preliminary, the only one conducted in Argentina to date.

Fourteen patients who had been selected but refused to undergo specific therapy and whose unfavorable course has come to our attention, might be considered the control group.

During the first 90-day period, all patients from the selected group were treated with doxycycline plus tetracycline sulfate. In a second 90-day period, therapy was carried out with ceftriaxone plus Nefazodone. In some cases olanzapine was added. Nefazodone was replaced by Fluoxetine plus olanzapine, or by sertraline plus olanzapine. In a third 180-day period, antibiotic therapy was changed to benzathine penicillin plus azithromycin.

Vancomycin, a last-option drug, was given to only 2 patients who were refractory to other antibiotics.

Prior to antibiotic administration, patients underwent IgE RAST and blast transformation tests for allergy risk. Levotiroxine was added for patients with hormonal tests revealing T4 reaching boundary or lower values. Complementary therapies of multivitamins, acidophilus preparations, oligo-elements, and essential fatty acids were prescribed. Physical rehabilitative therapy was recommended. Alcohol, coffee, and smoking were prohibited.

**Laboratory tests**

The IgG and IgM response to *B. burgdorferi* was determined in all patients' sera with indirect immunofluorescence assay (IFA). Samples were further examined for DNA specific for *Borrelia burgdorferi* sensu lato by nested polymerase chain reaction (PCR), which included 3 specific Lyme-disease-causing species (*B. burgdorferi sensu stricto, B. burgdorferi afzelii, and B. burgdorferi garinii*).

**RESULTS**

IgG serology tests were positive for all patients. IgM antibodies were detected in only 2 cases. Laboratory test results were assigned 4/10 diagnosis criteria value. The decision was made to treat patients with antibiotic therapy concurrently with psychopharmacological therapy. (Table 1).

Concurrent with the antibiotic therapy, patients received psychiatric medication. (Table 2).

Previously, psychopharmacological therapies with identical psychotropics that had been proven to be inadequate or ineffective, were now accompanied by the specific antibiotics for Lyme disease and were highly effective and produced no side effects. Psychiatric symptomatology lessened and even disappeared. Atypical depression, only responsive to tetracycline promethazine, could be medicated with state-of-the-art psychotropics that have minimal therapeutic risks. Panic attacks disappeared and hypochondriacal symptoms diminished. Improvements in the varied associated clinical polysymptomatology were also observed under this new pharmacological approach. (Table 3).
**Table 1. Antibiotic therapy.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 90-day period 43*</td>
<td>Doxycycline oral (200 mg/d) 5 days in a row plus pulse therapy (400 mg/d) 2 days in a row.</td>
</tr>
<tr>
<td>Second 90-day period 17*</td>
<td>Ceftriaxone IM (2 g/d) 5 days in a row; 2-day rest to prevent lithiasic pathology risk.</td>
</tr>
<tr>
<td>Third 180-day period 9**</td>
<td>Benzathine penicillin IM (2 400 000 U) once weekly plus azithromycin (500 mg/d) pulse therapy 3 days in a row; 7-day rest. Total 27 intakes.</td>
</tr>
<tr>
<td></td>
<td>Benzathine penicillin IM (2 400 000 U) once weekly plus azithromycin (500 mg/d) pulse therapy 3 days in a row, 7 day rest. Total 27 intakes. During 1 relapsing episode total medication replaced by vancomycin IV (1000 mg/d) 10 days in a row.</td>
</tr>
</tbody>
</table>

*Oral therapy was accompanied by daily intake of acidophilus preparations. Alcohol and excessive caffeine contraindicated, starch and carbohydrates limited. 
**Prior to antibiotic change, patients underwent IgE Rasti and blastic transformational tests for penicillin allergy. Levotiroxine (0.1 mg to 0.2 mg/d) was added to patients with hormonal tests revealing T4 reaching boundary or lower values.

**Table 2. Psychopharmacological therapy.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 90-day period 43</td>
<td>Tranlypromine sulfate (20 mg/d).</td>
</tr>
<tr>
<td>Following periods 43</td>
<td>Nefazodone (100 mg to 200 mg/d) beginning with 50 mg daily increasing 50 mg every week) plus olanzapine (5 mg/d).</td>
</tr>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td></td>
<td>Fluoxetine (20 mg/d) plus olanzapine (5 mg/d).</td>
</tr>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td></td>
<td>Sertraline (50 mg/d) plus olanzapine (5 mg/d).</td>
</tr>
</tbody>
</table>

4. The psychiatric office met patients ultimate consultation result. Previous visits to numerous medical offices of diverse specialties rendered either no diagnosis or misdiagnosis, few improvements, and many relapses.

5. The antibiotic therapy received, radically proved ineffective. By ignoring the possibility of presumptive Lyme disease, Borrelia reproductive cycles were not taken into consideration. Patients given antibiotic therapy for clinical symptoms suffered from either an inadequate choice of antibiotics or a premature cessation of therapy, or both.

6. Psychiatric symptoms did not mean an actual basic psychiatric pathology but rather a symptomatology arising from presumptive Lyme disease.

**DISCUSSION**

Polymerase chain reaction tests in plasma with primers for the B burgdorferi sensu lato group were negative. This may be caused by: 1) patients not in the acute stage of the infection, spirochetemia was not present; 2) spirochetes were genotypically different from those described to date; and 3) it was not Lyme disease.

Without the fulfillment of Koch’s postulate, it is a challenge within an area not considered endemic to justify and prove the existence of the disease that we are describing.

On October 15, 1998, members of our research team from the Centro de Tecnología Salud Pública de la Universidad Nacional de Rosario, Argentina, reported the following to the World Health Organization (WHO) on “Suspected Lyme Disease in Argentina.”

1. Family groups were highly cooperative with their patient members and identified their needs and demands; no annoyance, tiredness, or lack of interest was found among these family groups were manifested.

2. Countertransferrential reaction was noticeably positive—the psychiatrist felt the need to help.

3. Patients with panic attacks never manifested derealization or depersonalization, neither did they feel fear of losing control or “going crazy.”

**CONCLUSION**

The possibility of administering specific state-of-the-art psychiatric medication replacing traditional therapy on 43 patients with atypical depression, panic attacks, and hypochondriasis, associated with multisystemic symptomatology is presented.

We also reported the effectiveness of combination anti-Lyme disease therapy and specific psychotropic therapy for probable Lyme disease.

**ACKNOWLEDGMENT**

This investigation was conducted in Rosario, Argentina, South America.

**REFERENCES**


2. Classification F41.0/F40.01 as per CIE-10. Classification 300.01/300.21 as per CIE-9-MC. DSM IV Spanish edition, 402:412, 1995.

3. Classification F45.2 as per CIE-10. Classification 300.7 as per
<table>
<thead>
<tr>
<th></th>
<th>Light Improvement</th>
<th>Significant Improvement</th>
<th>Complete Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>General clinic</td>
<td>—</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>Cardiology</td>
<td>3</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Dermatology</td>
<td>—</td>
<td>—</td>
<td>22</td>
</tr>
<tr>
<td>Endocrinology</td>
<td>—</td>
<td>—</td>
<td>32</td>
</tr>
<tr>
<td>Gastroenterology</td>
<td>—</td>
<td>—</td>
<td>43</td>
</tr>
<tr>
<td>Gynecology</td>
<td>—</td>
<td>—</td>
<td>24</td>
</tr>
<tr>
<td>Neurology</td>
<td>4</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>—</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Otorhinolaryngology</td>
<td>—</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>Rheumatology</td>
<td>8</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Urology</td>
<td>4</td>
<td>18</td>
<td>5</td>
</tr>
</tbody>
</table>

Photographic Section—An International Collection of Ticks: Part I

**THE MADAGASCAR TORTOISE TICK**

*Amblyomma chabaudi*, Rageu 1964; male (left) and female (right). Removed from several radiated tortoises (*Geochelone radiata*) and spider tortoises (*Pyxis arachnoides*) between December, 1998 and March, 1999 in the Cap Sainte Marie Nature Reserve, Province of Tulear by Thomas E.J. Leuteritz and Rollande Rovolanaivo. Identified by Richard G. Robbins, Walter Reed Army Medical Center, Washington, DC; submitted and photographed by James L. OCCI. Scale is in mm. Note: common name is unofficial.

**THE RHINOCEROS DERMACENTOR**

*Dermacentor rhinocerinus* (Denny, 1843); female (left) and male (right). Collected in burned grass savannah in Garamba National Park, Democratic Republic of Congo in 1951 by H. D. Saeger (RML No 37398). Supplied by James E. Keirans, U. S. National Tick Collection, Statesboro, Georgia, USA; submitted and photographed by James L. OCCI. Scale is in mm. See: Durden, LA and JE Keirans (1998). Host-parasite coextinction and the plight of tick conservation. *American Entomologist*, pp. 87-91 (Summer). Note: common name is unofficial.
THE ELEPHANT DERMACENTOR

Dermacentor circumguttatus Neumann, 1897; female (left) and male (right). Removed from Loxodonta africana (African elephant), Democratic Republic of Congo in 1998 by William B. Karesh, Wildlife Conservation Society, Bronx, NY. Identified by Richard G. Robbins, Walter Reed Army Medical Center, Washington, DC; submitted and photographed by James L. Occi. Scale is in mm. Note: common name is unofficial.

THE EAST AFRICAN BUFFALO TICK

Amblyomma cohaerens Dönitz, 1909; female (left) and male (right). Removed from Syncerus caffer (African Cape buffalo), Democratic Republic of Congo, March 1994, by William B. Karesh, Wildlife Conservation Society, Bronx, NY. Identified by Richard G. Robbins, Walter Reed Army Medical Center, Washington, DC; submitted and photographed by James L. Occi. Scale is in mm. Note: common name is unofficial.

James L. Occi
Biology Department
Union County College
Cranford, New Jersey
INFORMATION FOR CONTRIBUTORS

Journal of Spirochetal and Tick-borne Diseases

Dedicated to science and art in spirochetal and tick-borne diseases

INFORMATION FOR AUTHORS AND EDITORIAL POLICY

The following guidelines are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" and the International Committee of Medical Journal Editors (the "Vancouver Group") statement, agreed at the January 1993 Meeting.

The Journal of Spirochetal and Tick-borne Diseases publishes quarterly reviews and original work studies about any aspect of spirochetal and tick-borne diseases. The primary purpose is to broaden our understanding of spirochetal and tick-borne diseases. Special focus is given to Lyme borreliosis (also known as Lyme disease), as the most prevalent spirochetal and tick-borne disease. The clinical topics may involve all medical disciplines, nursing, and pharmacy, as well as the social, ethical, and biological features of spirochetal and tick-borne diseases.

The Journal is composed of two major sections. One section is devoted to the review of a specific topic that is established by the Associate Editors, and a special guest editor is invited to coordinate the development of up to six manuscripts relating to the specific topic. The second section of original works is composed of unsolicited manuscripts that are subsequently reviewed by the Review Board, as well as external reviewers, depending on the potential for conflict of interest within the editorial panel and the potential interest by the readership.

Expeditious review of all manuscripts is carried out with a projected response time of not more than 6 weeks. Rejected manuscripts usually are returned to authors within 6 weeks. Decisions about potentially acceptable manuscripts may take somewhat longer.

The Journal will publish material defined within the categories described in the following.

Reviews
Each issue includes a series of state-of-the-art articles on a topic related to spirochetal and tick-borne diseases. The articles represent invited presentation by authorities in the field on topics related to spirochetal and tick-borne diseases, with an emphasis on Lyme borreliosis.

Each manuscript should present a comprehensive state-of-the-art analysis and should be accompanied by an abstract of 300 words or less summarizing major points.

Peer Review Articles
Original articles of 5000 words or less may be submitted to the editorial office. Each article should be accompanied by an abstract of 300 words or less describing the findings of the original research. All articles will be peer reviewed within a 3-week period with subsequent notification to the authors within 5 weeks of submission. Submitted articles may relate to any area of spirochetal and tick-borne diseases.

Case Reports
Specific clinical case reports describing a unique approach to Lyme disease and other related disorders in the area of diagnosis or treatment may be submitted for review. An abstract of 250 words or less should accompany the text.

Correspondence
Letters to the Editor in the form of correspondence related to material published in the Journal or some aspect of Lyme borreliosis and other spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the Journal, will be referred to the author of the original work for a response. Letters to the Editor should be submitted in duplicate, typewritten and double-spaced, not exceeding 400 words of text and a maximum of five references. Letters should have no more than three authors, and should be signed by all of the authors. Please include a word count. Receipt of letters is not acknowledged, but correspondents will be notified when a decision regarding publication is made.

Editorials
Editorials may be published, usually at the solicitation of the Associate Editors, but unsolicited submissions that relate to an unusual topic of interest exceeding the usual designation of correspondence, i.e., 1000 words or less, will be considered.
Photographic Section

The topical photographic section will be a regular feature. Photographs pertinent to articles presented in the Journal, as well as other photographs related to any aspect of spirochetal or tick-borne diseases, will be considered for the publication. The guidelines for the submission are designated in Illustrations.

Conflict of Interest

The Journal asks authors of research articles to disclose at the time of submission any financial or other arrangements they may have with a company whose product figures in the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, the editors usually will discuss with the authors the manner in which such information is to be communicated to the reader.

Because the essence of reviews and editorials is selection and interpretation of the literature, the Journal expects that authors of such articles will not have any financial or other interest in a company (or its competitor) that makes a product discussed in the article. Potential authors who have questions about these issues should contact the Managing Editor.

Submission of Manuscripts

An original and three copies of the manuscript should be submitted to:

Journal of Spirochetal and Tick-borne Diseases
SLACK Incorporated
6900 Grove Road
Thorofare, NJ 08086

Manuscripts containing original material are accepted with the understanding that neither the article nor any part of its essential substance has been or will be published or submitted for publication elsewhere before appearing in the Journal.

All manuscripts should be accompanied by a letter of copyright transmittal. This must be signed and dated by all authors. The letter is required before any manuscript can be considered for publication and should contain the following wording:

"In consideration of The Lyme Disease Foundation taking action in editing my (our) submission, the author(s) undersigned hereby transfers, assigns, or otherwise conveys all copyright ownership to The Lyme Disease Foundation. The copyright so conveyed includes any and all subsidiary forms of publication, such as electronic media. The author(s) declares that the manuscript contains no matter that is, to the best of the author's knowledge, libelous or unlawful, or that infringes upon any U.S. copyright."

All manuscripts should be submitted with a cover letter indicating the category for which the manuscript should be reviewed. Copies of any closely related manuscripts should be submitted to the Editor along with the manuscript that is to be considered by the journal. A cover letter, signed by all authors, should identify the person (with the address and telephone number) responsible for negotiations concerning the manuscripts; the letter should make it clear that the final manuscript has been seen and approved by all authors and that they have taken due care to ensure the integrity of the work. Manuscripts should include a title page, abstract, and text, with tables, illustrations, and references below. For the integrity of the published material, manuscripts describing clinical aspects of Lyme borreliosis must disclose: criteria for patient enrollment into the study and criteria for defining "successful" or "non-successful" Lyme borreliosis treatment. Manuscripts without these requirements will be automatically rejected.

Titles and Author’s Names

With the manuscript, provide a page giving the title of the article; titles should be concise and descriptive (not declarative). Also include a running head of fewer than 40 letter spaces; the name(s) of the author(s), including the first name(s) and academic degree(s); the name of the department and institution in which the work was done; the institutional affiliation of each author; and the name and address of the author to whom reprint requests should be addressed. Any grant support that requires acknowledgment should be mentioned on this page.

Abstract

Provide on a separate page an abstract of not more than 300 words (original and review articles) or 250 words (case report). This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed, the results, and what the authors conclude from the results.

Text

All material should be typed and double-spaced. Standard sequence of methods and materials, results, and discussion should be employed with tables and figures numbered in the order in which they are cited in the text.

Tables

Submit tables typed and double-spaced and provide a heading for all columns with a comprehensive title on separate sheets.

Illustrations

Photographs and figures should be
submitted as glossy prints 5×7 in.,
with one copy of each print for each
copy of the manuscript. Figure leg-
ends should be provided on a sepa-
rate sheet with identification of the
figure. The back of the glossy print
should indicate the number of the fig-
ure.

References
References should be numbered
in order of citation in the text, fol-
lowing the American Medical
Association guidelines for refer-
ences. The standard journal abbrevi-
atations from Index Medicus should be
followed. Numbered references to
personal communications, unpub-
lished data, and manuscripts either
“in preparation” or “submitted for
publication” are unacceptable.

Drug Names
Generic names generally should
be used. When proprietary brands are
used in research, include the brand
name in parentheses in the Methods
section.

Units of Measure
Authors should express all mea-
surements in conventional units,
with Système International (SI)
units given in parentheses through-
out the text. Figures and tables
should use conventional units, with
conversion factors given in legends
or footnotes.

Permissions
Materials taken from other
sources must be accompanied by a
written statement from both author
and publisher giving permission to
the Journal for reproduction. Obtain
permission in writing from at least
one author of articles still in press,
unpublished data, and personal
communication. Contributors are
obligated to disclose that submis-
sions constitute their own work
unless otherwise specified. Contribu-
tors shall indemnify the
Journal for any claims or actions
instituted against the Journal by
individuals claiming that the con-
tribution is not the work of the con-
tributor.

Acknowledgment of Receipt
An acknowledgment, with a re-
ference number for future inquiries,
is dispatched immediately (this does
not apply to letters).
Mothers.
Spouses. (maybe)
Medical Matrix.

What do they have in common? You place your trust in them. When searching the Internet for credible medical information, you need a resource you can trust. The Lancet quotes that “Medical Matrix is the most comprehensive compendium of reliable medical information on the Internet.”

MEDICAL MATRIX LLC
http://www.medmatrix.org

The largest, peer-reviewed directory to the medical Internet.
Free registration.