Flying Squirrel-Associated *Rickettsia prowazekii* (Epidemic Typhus Rickettsiae) Characterized by a Specific DNA Fragment Produced by Restriction Endonuclease Digestion

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The DNA from flying squirrel-associated *Rickettsia prowazekii* was characterized by using a specific DNA fragment produced by digestion with the enzyme *Bam*HI. The DNA fragment was cloned into a plasmid vector and used to readily distinguish between available human- and flying squirrel-associated *R. prowazekii* DNAs derived from crude cytoplasmic extracts.

Sporadic human typhus associated with *Rickettsia prowazekii*-seropositive flying squirrels (*Glaucomys volans*) is well documented in the eastern United States (1, 7). No isolates of the infectious agent have yet been obtained from humans. The spread of disease beyond the primary case in a human to other humans has not been reported in the United States, probably because of the scarcity of a competent vector. However, flying squirrels inhabit the same geographic areas as some human populations that may be infected with the human body louse, *Pediculus humanus* (the recognized vector of classic human epidemic typhus), and this louse is an efficient experimental vector for *R. prowazekii* isolated from flying squirrels (2). The distribution of *G. volans* and the closely related species *G. sabrinus* extends as far south as Honduras in the western hemisphere (5); the monophyletic genera of flying squirrels are circumpolar (11). Thus, the possibility exists that human epidemics of louse-borne typhus could be initiated from flying squirrel sources. The inability to distinguish reliably and easily among various isolates of *R. prowazekii*, however, has not allowed for a complete evaluation of the origins of human typhus infections.

Slight differences between flying squirrel *R. prowazekii* isolates and *R. prowazekii* isolates from other sources have been reported. These differences include a shift in isoelectric focusing characteristics of a single polypeptide (4) and slight differences in *Sac*I restriction endonuclease DNA fragment patterns that could easily be confused with the *Sac*I DNA fragment patterns of other *R. prowazekii* isolates (9). The electrophoretic mobility of a large polypeptide from flying squirrel and other *R. prowazekii* isolates has been reported to be selectively heat modifiable (G. A. Dasch, J. P. Burans, M. E. Dobson, R. I. Jaffe, and W. G. Sewell, 3rd Int. Symp. Rickettsiae and Rickettsial Dis., Smolenice, Czechoslovakia, 1984, p. 4).

In this communication we report that *R. prowazekii* isolates from southern flying squirrels (*G. volans*) can be very readily differentiated from available human isolates of *R. prowazekii* from Europe and Africa by *Bam*HI restriction endonuclease analysis of rickettsial DNA. Isolates of *R. prowazekii* from New World human sources were not avail-

able for testing. Cultivation and purification of rickettsiae, as well as subsequent extraction of rickettsial DNA and restriction endonuclease analysis, were done as previously described (9).

Wood et al. (12) recently reported that cleavage of rickettsial DNA with restriction endonuclease *Bam*HI can be used to differentiate the Madrid E and Breinl isolates of *R. prowazekii*. The data in Fig. 1 show that the *Bam*HI cleavage pattern of *R. prowazekii* Madrid E is unique among those of the several *R. prowazekii* isolates tested; note the appearance of the *R. prowazekii* Madrid E DNA fragment migrating at a position comparable to the 9.4-kilobase-pair (kbp) lambda *Hind*III DNA fragment marker. Data presented elsewhere (R. L. Regnery and C. L. Spruill, 3rd Int. Symp. Rickettsiae and Rickettsial Dis., Smolenice, Czechoslovakia, 1984, p. 4) showed that the unique DNA fragment found in *Bam*HI digests of *R. prowazekii* Madrid E is the result of an additional *Bam*HI site in the DNA of the Madrid E isolate and not the result of large detectable changes in the genetic composition of *R. prowazekii* Madrid E.

When the same restriction endonuclease, *Bam*HI, was used for digestion of rickettsial chromosomal DNA from flying squirrel *R. prowazekii* isolates, a DNA fragment with a unique electrophoretic mobility was observed (Fig. 1). The estimated size of the diagnostic fragment is 3.7 kbp. A DNA fragment with this mobility was not found in any of the *Bam*HI digests of five representative *R. prowazekii* isolates from Old World human epidemic typhus specimens.

To facilitate future applications of the observation that the DNA from flying squirrel *R. prowazekii* isolates has a readily detectable genetic marker, we investigated the use of a specific cloned DNA probe for differentiation of flying squirrel and human *R. prowazekii* isolates. The *Bam*HI-cleaved, 3.7-kbp diagnostic DNA fragment from flying squirrel isolate F-12 was enzymatically inserted into the *Bam*HI cloning site of plasmid pUC-19; the plasmid-rickettsia DNA sequence was then greatly amplified in the *Escherichia coli* host (strain TB1) and purified on ethidium bromide-caesium chloride isopycnic gradients (6). To demonstrate the diagnostic character of the cloned DNA, we isotopically labeled the DNA in vitro with [³⁵S]dATP and DNA polymerase I and then hybridized it with *Bam*HI digests of *R. prowazekii* chromosomal DNAs from both human and flying squirrel isolates (Fig. 2). The cloned probe allowed for unambiguous differentiation between the *Bam*HI digests of *R. prowazekii* DNAs from flying squirrel and human isolates. The probe

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FIG. 1. Agarose gel electrophoresis of *Bam*HI-digested *R. prowazekii* DNAs from human and flying squirrel isolates. *Bam*HI digests of rickettsial DNA were subjected to electrophoresis in 0.6% agarose gels as previously described (9). The *R. prowazekii* isolates from flying squirrels were F12 (Florida), F16 (Florida), V250 (Virginia), and V257 (Virginia). The *R. prowazekii* isolates from humans were Breinl (Poland), Jaf (recrudescent infection of a Russian immigrant), Madrid E (Spain), Addis Ababa (Ethiopia), and Cairo (Egypt). The complete histories of the *R. prowazekii* isolates are given elsewhere (1, 3, 8, 9). DNA fragments of bacteriophage lambda cleaved with *Hind*III were used as size standards; the sizes of the fragments are given in kilobase pairs to the left of the figure (10). The arrow indicates the position of the flying squirrel-specific 3.7-kbp DNA fragment.

appeared to hybridize to much larger *Bam*HI DNA fragments in digests of DNA from human *R. prowazekii* isolates, suggesting that DNA sequences similar to the 3.7-kbp diagnostic DNA fragment were present in all *R. prowazekii* genomes tested (but not in that of *R. rickettsii*). Hybridization of isotopically labeled rickettsial chromosomal DNA to digests of the 3.7-kbp fragment made with restriction enzymes other than *Bam*HI indicated that the appearance of the 3.7-kbp fragment was the result of an additional *Bam*HI recognition site found in the DNA of flying squirrel *R. prowazekii* isolates (data not shown).

To minimize the cultivation and purification of rickettsiae required for DNA analysis, we made cytoplasmic extracts from minimal amounts of rickettsia-infected Vero cells (as little as a single 150-cm² cell culture vessel per rickettsial isolate) with 0.25% Triton X-100 and removed the host cell nuclei by low-speed centrifugation. These relatively crude extracts of cytoplasmic DNA were phenol-chloroform extracted, ethanol precipitated, and suspended in Tris buffer (10 mM, pH 8.0). The cytoplasmic extracts containing rickettsial DNA were suitable for hybridization analysis with the cloned probe (Fig. 2).

Only isolates of *R. prowazekii* from *G. volans* from the eastern United States have been examined. Before generalizations can be drawn regarding the observed differences in *Bam*HI DNA digestion patterns, it will be necessary to examine typhus isolates from geographically distinct populations of flying squirrels. It should be clear, however, that simplified technological means are now available to begin to provide data that can be used to draw definitive conclusions regarding the relationship between isolates of *R. prowazekii*

FIG. 2. Clonal DNA differentiation between flying squirrel and human *R. prowazekii* isolates. Various preparations of rickettsial DNA were subjected to electrophoresis in a 1% agarose gel, the DNA was blotted to nitrocellulose, and the blotted DNA was probed at 63°C with the 3.7-kbp, ³⁵S-labeled probe (6). Only DNA from flying squirrel *R. prowazekii* isolates hybridized with the labeled probe in the 3.7-kbp region. The specific hybridization pattern was the same for DNAs from both purified organisms (purified rickettsiae) and cytoplasmic extracts (cyto. extr.) of rickettsia-infected cells. R.r., DNA extracted from *R. rickettsii*-infected cell cytoplasm. The *Hind*III digest of bacteriophage lambda DNA that was used for size standards was isotopically end labeled in vitro with [³⁵S]dATP and the Klenow fragment of DNA polymerase I (9). The sizes of the standard DNA fragments are given in kilobase pairs to the left of the figure (10).

from flying squirrels and any isolates from future human typhus infections or human typhus epidemics. Such information is essential if the global eradication of human epidemic typhus is ever to be seriously considered.

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