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 BamHI. 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 and the finding 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 SacI restriction endonuclease DNA fragment patterns that could easily be confused with the SacI 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 BamHI restriction endonuclease analysis of rickettsial DNA. Isolates of R. prowazekii from New World human sources were not available 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 BamHI can be used to differentiate the Madrid E and Breinl isolates of R. prowazekii. The data in Fig. 1 show that the BamHI 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 HindIII 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 BamHI digests of R. prowazekii Madrid E is the result of an additional BamHI 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, BamHI, 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 BamHI 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 BamHI-cleaved, 3.7-kbp diagnostic DNA fragment from flying squirrel isolate F-12 was enzymatically inserted into the BamHI 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-cesium 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 BamHI digests of R. prowazekii chromosomal DNAs from both human and flying squirrel isolates (Fig. 2). The cloned probe allowed for unambiguous differentiation between the BamHI digests of R. prowazekii DNAs from flying squirrel and human isolates. The probe

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appeared to hybridize to much larger BamH I 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 BamHI indicated that the appearance of the 3.7-kbp fragment was the result of an additional BamHI 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 BamH I 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 from flying squirrels and any isolates from future human typhus infections or human typhus epidemioms. Such information is essential if the global eradication of human epidemic typhus is ever to be seriously considered.

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LITERATURE CITED