Letters to the Editor

Evidence for an Association between JC Virus and Colorectal Neoplasia

To the Editor: Newcomb et al. (1) have attempted to confirm the presence of JC virus (JCV) DNA in colorectal cancers and have failed to do so. They reached their negative conclusion, although other groups have been able show JCV in colonc epithelium and colorectal neoplasia (2-4). Whether this polyomavirus, which encodes a potent oncogene (T antigen), causes colon cancer remains an open question. However, its presence in colonic tissues and colorectal neoplasms in particular is not a question. This has been reported convincingly from two laboratories.

Our group has repeatedly confirmed this observation on different specimens of colorectal cancer (published and unpublished), each time using multiple controls to ensure the absence of PCR contamination. The assay is somewhat technically difficult and requires a focused effort by a dedicated laboratory to perfect. We began work on this project in 1994, and although the initial experiments were positive, we took several months to optimize the PCR conditions. JCV is a small circular DNA virus and may be present in relatively small amounts in colorectal neoplasms. Newcomb et al. used novel primers and an entirely different method for their assays. Specifically, all of their assays were done on DNA extracted from formalin-fixed, paraffin-embedded specimens. We initially had some difficulty performing JCV PCR from paraffin. The formalin fixation breaks DNA and may make it more difficult to recover sufficient quantities of intact JCV DNA for amplification. Newcomb et al. may have had difficulty with PCR amplification of JCV out of paraffin as well. In fact, they reported no positive controls in which they were able to get this PCR to work from paraffin tissues. They readily obtained PCR products from a plasmid preparation or urine samples, much the same way we have readily obtained PCR evidence for JCV from frozen specimens that have never been fixed in formalin. The only positive PCR result obtained from the 233 fixed tissues by Newcomb et al. was not confirmed either by Southern blot or DNA sequencing; even this may have been a nonspecific result.

Essentially, Newcomb et al. have attempted to use an assay that had not undergone appropriate, relevant validation and speculated that their failure to obtain PCR products refutes multiple lines of evidence from other laboratories. A BLAST analysis indicates that the T-antigen primers in this study form multiple primer dimers, which would almost certainly limit the PCR efficiency. Moreover, other primers were found to have complete sequence matches with BK virus as well as JCV, suggesting that the primer design was not optimal.

It is unlikely that the results from our laboratory represent assay contamination because of the DNA sequence variations we have found in the T antigen and in the transcription control region. Contamination is likely to provide a uniform product. Moreover, it is extremely unlikely (and would be unprecedented) that the rearrangements in the transcription control region reported by Ricciardiello et al. (5) represent PCR artifacts, as these sequence alterations are additions or deletions of a 98-bp cassette; similar rearrangements are generated during viral replication in nature and have been found in human samples by others (6, 7). The authors’ suggestion that our PCR amplification of JCV represents nonspecificity due to the use of degenerate primers makes little sense, as our PCR products were validated both by Southern blot and by DNA sequencing. In addition, Enam et al. (4) have confirmed our findings, and extended these, by demonstrating protein expression of the T antigen and agnoprotein in colon cancers, but not normal colon, using immunohistochemistry. This important confirmation was dismissed without consideration by Newcomb et al. Considering the work of two independent laboratories, it is difficult to accept that a novel, unvalidated assay is sufficient to conclude that the prior investigators have been inaccurate.

It is reasonable for Newcomb et al. to conclude that they are unable to document the presence of JCV using their approach on paraffin-embedded tissues. The assay developed by our group and Khalili’s group took considerable effort to validate. The best explanation for Newcomb et al.’s findings is that they have not made the assay sufficiently sensitive to find JCV DNA sequences that are actually present.

C. Richard Boland, M.D.
Division of Gastroenterology
Baylor University Medical Center
Dallas, Texas

References
variants with distinctive regulatory sequences in the brain of a
single patient with progressive multifocal leukoencephalopathy.

In Response: Dr. C. Richard Boland raises concerns
about the ability of our study to detect an associa-
tion between JC virus and colorectal cancer. Our data
do suggest that it can be present in colon, albeit at
much lower frequency than his group (1, 2) and
Enam et al. (3) report. However, in our opinion,
either their data nor ours support a role of JC virus
as a cause of colon cancer (4). Ricciardiello et al. (2) re-
port a high prevalence of JC virus in gastric and colonic
tissue from patients without gastrointestinal neoplasia
(70% of participants). In our study, the prevalence of
JC virus was <0.005% in normal tissue from colorectal
cancer patients.

The cause of the large difference in JC virus prevalence
probably is due to technical issues. Blast searches done
at the time we designed the primers as well as Blast
searches done now did not indicate homologies between
our PCR primers and BKV sequences, as suggested by
Dr. Boland. Our primers and probes were designed
using PrimerExpress (Applied Biosystems, Foster City,
CA), which eliminates primers pairs that are likely to
form dimers.

In our assay design, we did take into account the
lower quality of DNA from paraffin-embedded tissue
we were testing by designing shorter amplicons (100–150
bp), which amplify well even from degraded DNA. As
we explained in the Discussion section of our article,
we had no problems amplifying 10 markers of similar
size in single-copy genes from these DNAs and have no
reason to believe that the DNA contained contaminants
that inhibited amplification. We also screened DNA from
normal, fresh frozen colon biopsies from 80 indivi-
duals, none of which turned out to be JC virus positive.
Furthermore, their report that Mad-1 is the exclusive
JC virus strain found in the human colon (5) is not
consistent with our finding of a colonic isolate that did
not have a rearranged regulatory region. This fact was
not stated clearly enough in our article and we are
grateful to Dr. Boland for an opportunity to make up
for this omission.

Our criteria for a “JC virus positive” call were more
stringent than those of Laghi et al. (1). Samples were
classified positive only if we could obtain sequence for at
least two of the three fragments tested, whereas in Laghi
et al. (1) one positive amplification of an average of four
amplifications per sample led to a positive call. This dif-
fERENCE alone would have an effect on the proportion
of positive calls.

Dr. Boland argues that the presence in their study of
different JC virus sequences within an individual
speaks against contamination with their positive con-
trol. Although it is well known that Taq polymerase is
error prone (6), this is not usually a problem when
direct sequencing PCR products but will become notice-
able when cloning PCR products and then sequencing
individual clones, especially when starting with a
small copy number of template (6). In such a case, it
is essential to sequence numerous clones to obtain a
consensus sequence. Thus, the sequence differences in
cloned derived from the same patient as reported by
Dr. Boland’s group (1, 2, 5) are much more likely due to
errors introduced during PCR amplification than intra-
individual variation. Unfortunately, direct sequencing
of some of their PCR fragments does not seem possible
because of other fragments that are coamplified in their
PCRs (see figures in refs. 1, 2, 7). Our amplifications,
however, were optimized to allow direct sequencing,
which is preferable to Southern hybridization as a tool
for sequence verification. We agree that the 98-bp dele-
tion in their clones is not likely due to PCR artifacts.
However, because this region is prone to rearrangements,
it is very conceivable that such rearrangements occur
while growing up the clones. A comparison of direct
sequencing of the PCR fragment before cloning and
sequencing individual clones afterward could address
this question.

In summary, the reports from Dr. Boland’s group had
some important limitations. The intraindividual se-
quency heterogeneity on the one hand and the finding
of the same strain in different individuals on the other (5)
are not consistent with other published work: (a) longitudi-
nal studies that show shedding of the same JC virus
strain (8), (b) extensive work on the geographic distribu-
tion of different JC virus strains (e.g., ref. 9), and (c) and
uniqueness of rearrangements of neurotropic JC virus
isolates from different individuals (e.g., ref. 10).

Jeannette Bigler
Polly A. Newcomb
Johanna W. Lampe
John D. Potter

References
1. Laghi L, Randolph AE, Chauhan DP, et al. JC virus DNA is present in
the mucosa of the human colon and in colorectal cancers. Proc Natl
are frequently present in the human upper and lower gastrointes-
omavirus JCV with colon cancer: evidence for interaction of vir-

4. Blaho JA, Aaronson SA. Convicting a human tumor virus: guilt by
5. Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, Boland
CR. Mad-1 is the exclusive JC virus strain present in the human
colon, and its transcriptional control region has a deleted 98-
6. Saiki RK. The design and optimization of the PCR. In: Erlich HA,
editor. PCR technology: principles and applications for DNA amplifi-
mosomal instability in colonic cells by the human polyomavirus
infection is demonstrated by continuous shedding of the same JCV
9. Agostini HT, Jobes DV, Stoner GL. Molecular evolution and
epidemiology of JC virus. In: Khullar K, Stoner GD, editors. Human
polyomaviruses: molecular and clinical perspectives. Hoboken (NJ):
10. Agostini HT, Ryschkewitch CF, Singer EJ, Stoner GL. JC virus
regulatory region rearrangements and genotypes in progressive multi-

focal leukoencephalopathy: two independent aspects of virus variation.
Evidence for an Association between JC Virus and Colorectal Neoplasia

C. Richard Boland


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/13/12/2285

Cited articles
This article cites 11 articles, 8 of which you can access for free at:
http://cebp.aacrjournals.org/content/13/12/2285.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/13/12/2285.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.