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LETTERS TO THE EDITOR

Re: Cottliar *et al.*: High Frequencies of Telomeric Associations, Chromosome Aberrations, and Sister Chromatid Exchanges in Ulcerative Colitis

TO THE EDITOR: We read with interest the article by Cottliar *et al*. in the September 2000 issue (1). In their study, the authors measured telomeric associations (TAs) in peripheral blood lymphocytes (PBLs) of ulcerative colitis patients as a parameter to evaluate chromosome instability. They contend that the mechanism that causes the TAs is not known, but cite work showing shortening of telomeres in ulcerative colitis as a potential mechanism (2).

There is evidence from the literature (3) that telomerase activity is decreased in patients with inflammatory bowel disease (IBD). This and other evidence (4) showing decreased capacity for DNA repair in IBD patients may prove to be the mechanism for TAs. In contrast to the above data showing decreased telomerase activity in IBD patients, increased telomerase activity is seen in a variety of cancers, including colon cancer (5). However, there is also a recent report showing that there may not be a direct relationship between colorectal cancer telomere length and telomerase activity (6), suggesting that this relationship may be quite complex. Our work on telomerase in colonic effluent has confirmed that telomerase activity is decreased in IBD (7). In contrast, our work in a spontaneous animal model of colitis and colorectal cancer, the cotton-top tamarin, which has an extremely high incidence of colorectal cancer following chronic colitis, shows higher telomerase activity in the effluent samples of tamarins as compared to humans with IBD. This suggests that telomerase activity may be a marker of colorectal cancer risk in the tamarin, but this is unlikely to be the case in humans, who have a relatively lower incidence of colorectal disease (8).

In addition, the concept of using PBLs for analysis of chromosomal instability should be re-examined. It is possible that chromosomal aberrations in PBLs may not represent similar damage in the target organ tissue because lymphocytes, primarily involved in the immunological reaction of IBD, could be damaged in the inflammatory process, resulting in chromosomal instability that may not be related to the increased colon cancer risk seen in ulcerative colitis. This has been shown to be the case with the PBLs of patients with Crohn's disease demonstrated to have chromosomal aberrations (9). Furthermore, it has been reported that chromosomal damage to PBLs may result from *in vitro* exposure to agents commonly used in the treatment of IBD (10). We therefore believe that the demonstration of chromosomal aberrations in the PBLs of IBD patients is a nonspecific epiphenomenon and that more specific tests are needed to demonstrate a causal association between chromosomal damage in PBLs and cancer risk in this scenario.

Rama Marepally, M.D.
Martin Tobi, M.B., Ch.B.
Gastroenterology Service
John D. Dingell VAMC
Center for Molecular Medicine and Genetics
Wayne State University School of Medicine
Detroit, Michigan

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Reprint requests and correspondence: Rama Marepally, M.D., Gastroenterology Service, John D. Dingell VAMC, 4646 John R. Street, Detroit, MI 48201-1932.

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Response to Drs. Marepally and Tobi

TO THE EDITOR: We read with interest the letter by Marepally and Tobi and welcome the opportunity to discuss

some points. The pathways that contribute to telomeric association (TA) formation are still a matter of debate. Different studies suggest that TAs may be formed either through association of telomeric single strand overhangs (1) or through telomere-binding protein associations (2). Moreover, a specific function is required to separate telomeres in mitosis, and this function is dependent on the presence of correct telomeric sequences or proteins that bind to these sequences (3). It is known that telomere shortening in human chromosomes usually leads to increased frequencies of TAs (4). Thus, when telomeres become critically short, telomere separation in mitosis cannot be performed properly, leading to telomeric fusions and chromosome instability. This karyotypic instability produced by telomeric fusions can be relevant to carcinogenesis because it increases the probability of errors that can generate genetic changes critical in the multistep process of transformation (5). Furthermore, deficient DNA repair was not proved to be a mechanism for TAs origin, even though it is another expression of genomic instability.

There is evidence that telomerase is the major mechanism for telomeric maintenance in most eukariotic cells (6). It has been suggested that a failure in telomerase activity may result in chromosomal stability leading to continuous cell proliferation (7). A direct correlation between telomeric shortening during the immortalization process and TA by a telomerase-dependent mechanism has been observed in human cell lines (8). Moreover, different telomerase-independent pathways have also been described (9). No telomerase activity has been reported in normal somatic cells. However, an increased activity was detected in a wide range of malignancies, whereas decreased levels were observed in patients suffering inflammatory bowel disease (IBD) as compared to colon carcinoma patients (10). This would suggest the existence of a gradient in telomerase activity, telomeric length, and TAs from health to the putative neoplasia, passing over an intermediate cancer prone condition such as ulcerative colitis (UC). Therefore, low or intermediate levels of telomerase activity in UC may be a finding that should be taken into account in cancer proneness. A similar situation was described in the progression from benign melanocytic cells to metastatic melanocytic cells (11).

In addition, the analysis of chromosomal aberrations (CAs) in peripheral blood lymphocytes (PBLs) is among the biomarkers most commonly used to evaluate chromosome instability and cancer risk. The idea of a causal association between CAs and cancer risk is based on the concept that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis. Recent epidemiological studies have contributed to the validation of CAs as an intermediate endpoint in carcinogenesis (12, 13). These studies support the idea that chromosome damage itself is involved in the pathway to cancer and suggest that individual characteristics such as polymorphisms of genes involved in carcinogen metabolism and DNA repair, genetic instability, and nutritional status could be related to the CA–cancer risk association.

Table 1. Correlation Coefficients Between Clinical Parameters of UC Patients and the Mean SCE for High Frequency Cell Frequencies

	HFC/Cell	
Clinical Parameters	*r*	*p*
Severity of disease*	0.32	NS
Duration of disease	0.23	NS
Disease extension*	−0.39	NS

NS = differences not significant, $p > 0.05$.

* Each patient of the disease severity and extension subgroups was assigned a value for the calculation coefficients (mild = 1, moderate = 2, and severe = 3); in the same way, distal = 1, left-sided = 2, and extensive = 3.

Moreover, as we wrote in our article, UC patients did not show significant correlations among the frequencies of CAs, TAs, and sister chromatid exchanges (SCE) and the clinical parameters evaluated (disease extension, disease activity, and evolution time). On the other hand, SCE studies in Crohn's disease (CD) have shown a significant correlation between the mean SCE for high frequency cell and the disease severity, suggesting an association with the inflammatory condition of CD (14). Marepally and Tobi suggest that a similar situation could be responsible for our results in UC. We include here the evaluation of high frequency cells in our UC patients; we did not find any correlation with clinical parameters (Table 1), indicating that UC has a different behavior than CD and supporting our initial suggestion about the association between the chromosome instability found in UC patients and the cancer predisposition observed in this disease.

Furthermore, it is necessary to clarify that even though the agents used in inflammatory bowel disease treatment induce *in vitro* chromosomal damage in PBLs, we have studied untreated patients with UC, and patients with smoking, alcohol, or any type of drug habits were excluded, as was established in our article. Therefore, we reaffirm that the increased chromosome instability observed in PBLs from UC patients is a specific phenomenon, not related to the immunological status, that reflects the presence of an unstable genome in these patients associated with the UC cancer predisposition.

Alejandra Cottliar, M.Sc.
Ariela Fundia, Ph.D.
Irma Slavutsky, Ph.D.
Departamento de Genética
Instituto de Investigaciones Hematológicas
"Mariano R. Castex"
Academia Nacional de Medicina
Buenos Aires, Argentina

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Reprint requests and correspondence: Alejandra Cottliar, M.Sc., Departamento de Genética, Instituto de Investigaciones Hematológicas "Mariano R. Castex," Academia Nacional de Medicina, Pacheco de Melo 3081, 1425 - Buenos Aires, Argentina.
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Are Minimicrosphere Pancrelipase Capsules Effective Enough for the Treatment of Pancreatic Steatorrhea?

TO THE EDITOR: We have a couple of comments regarding the article by Stern *et al*. (1). This study was to address the safety and efficacy of minimicrospheres, which are enteric coated, delayed release pancrelipase capsules, on fat absorption in pediatric/adolescent and adult cystic fibrosis patients. The authors' conclusion was that minimicrosphere pancrelipase capsules are an effective treatment for steatorrhea associated with pancreatic insufficiency in patients with cystic fibrosis. However, we think that caution is needed in interpreting the data and thus in reaching that conclusion.

Stern *et al*. conducted two randomized, placebo-controlled studies consisting of an open-label run-in phase followed by a double blind phase. The open-label run-in phase involved stabilizing all patients on pancrelipase minimicrospheres while the patients were on a high fat diet. Patients with a coefficient of fat absorption (CFA) of >80% were then randomly assigned either to continue to take pancrelipase minimicrosphere capsules or to receive placebos. The results of the double blind phase of the study showed a marked response to pancrelipase. It was stated in the discussion that it could be argued that this response occurred because they included only patients who responded (defined as CFA >80%) during the open label treatment phase in the double blind phase of the study. However, they asserted that because only 20% of the open label patient population (12 of 50 adults [24%] and 7 of 47 pediatric/adolescent patients [15%]) were nonresponders, pancrelipase would likely alleviate steatorrhea in the majority of cystic fibrosis patients with pancreatic insufficiency.

We do know well that the current therapy for the treatment of pancreatic steatorrhea including porcine pancreatic enzymes with or without antacids, histamine-2 receptor blockers, proton pump inhibitors, and enteric coating is effective in reducing the severity of steatorrhea (2). However, the problem is that the current therapy does not abolish it (3, 4). This fact is also reproduced in the study of Stern *et al*. (1). Twenty percent of their patients did not respond to pancrelipase minimicrosphere capsules. Moreover, are patients or physicians satisfied only if CFA is >80%? What does a CFA of 81% mean if a patient takes 100 g of fat a day? This means that the patient excretes 19 g of fat in feces a day. It is recommended that a pancreatic enzyme replacement therapy should be initiated if stool fat excretion exceeds 15 g/day (2). So we believe that the clinically acceptable lower limit of CFA is >85% (5), and we can say that steatorrhea is corrected if CFAs are ≥90%. If this definition is applied to the study by Stern *et al*. (1), a fair proportion of the patients would still be receiving unsatisfactory treatment with pancrelipase minimicrosphere capsules.

Stern *et al*. (1) did not mention the difference between minimicrospheres and microspheres/microtablets that are widely used, especially in Europe (2, 5). Minimicrosphere pancrelipase capsules should have been produced to enhance mixing of lipolytic activity with food in the lumen and thereby to increase fat absorption. Hence, it should have been more appropriate to compare the effects of minimicrospheres and microspheres/microtablets, although a negative study result is already available (6). Mixing of lipolytic activity with food is believed to play an important role in