Differential expression of EGFR during early reparative phase of the gastric mucosa between young and aged rats

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Liu, Lei, Jerrold R. Turner, Yingjie Yu, Ahmed J. Khan, Richard Jasiewski, Suzanne E. G. Fligiel, and Adhip P. N. Majumdar. Differential expression of EGFR during early reparative phase of the gastric mucosa between young and aged rats. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G943–G950, 1998.—Aging is associated with decreased reparative ability of the gastric mucosa. Our recent data suggest a role for epidermal growth factor receptor (EGFR) in the mucosal reparative processes. Thus we examined changes in EGFR tyrosine kinase activity as well as expression and subcellular localization of EGFR and its ligand transforming growth factor-α (TGF-α) in the gastric mucosa of young (4-mo-old) and aged (24-mo-old) Fischer 344 male rats during the early reparative phase after acute injury induced by 2 M NaCl. Within 240 min of injury, significant epithelial restitution was observed in the gastric mucosa of young but not of aged rats. Expansion of the neck region and initiation of foveolar cell migration could be seen within 45 min of injury in young rats but not until 90 min in aged rats. In young rats mucosal EGFR tyrosine kinase activity increased at 45 min after injury and subsequently fell to basal levels. Mucosal EGFR mRNA increased throughout the reparative phase as did content of the EGFR ligand TGF-α. In contrast, although the basal tyrosine kinase activity and levels of EGFR mRNA and TGF-α were elevated in the gastric mucosa of aged rats, injury did not cause increases in these parameters. Immunofluorescent localization suggests that internalization and/or degradation of EGFR may be higher in aged than in young rats. We suggest that diminished induction of EGFR tyrosine kinase activity and increased EGFR internalization after injury may in part be responsible for the age-related decrease in the reparative capacity of the gastric mucosa.

Results from this laboratory have demonstrated that aging is associated with diminished reparative ability of the gastric mucosa (5, 10, 13, 14). Although the regulatory mechanisms for the age-related decline in mucosal reparative ability remain to be fully elucidated, our recent observations suggest a role for epidermal growth factor receptor (EGFR) tyrosine kinase in regulating mucosal reparative processes (10, 11, 22). In evaluating the involvement of EGFR tyrosine kinase in the gastric mucosal restitutional process, we have observed that in young, but not in aged rats, gastric mucosal EGFR tyrosine kinase activity is greatly increased within 30 min after acute injury (22). This suggests that, in aged gastric mucosa, diminished activation of EGFR tyrosine kinase resulting in decreased activation of the EGFR signaling pathway after injury may partly be responsible for attenuation of the reparative process (10, 22). However, the regulatory mechanism(s) for the diminished activation of EGFR tyrosine kinase in aged rats during the initial reparative period is poorly understood. We postulate that transforming growth factor-α (TGF-α), which is synthesized in the gastric mucosa and is one of the primary ligands of EGFR (19, 30), may play a critical role in regulating EGFR tyrosine kinase activity during the initial mucosal reparative phase after acute injury. The basis for this postulation comes from the observation that levels of TGF-α in the gastric juice as well as in the gastric mucosa are substantially elevated in young rats within 30 min after acute injury (21). On the other hand, in aged rats, we found no significant change in mucosal TGF-α levels at 30 min after acute injury (22). To further evaluate the role of EGFR tyrosine kinase in the age-related decline in mucosal restitution, the current study examines changes in tyrosine kinase activity of EGFR as well as expression and subcellular distribution of the receptor in the gastric mucosa over a 240-min reparative period after acute injury. The significance of endogenous TGF-α in regulating EGFR tyrosine kinase during this reparative phase is also examined.

MATERIALS AND METHODS

Animals and collection of tissues. Male Fischer 344 rats, aged 4 and 24 mo old, were used. The animals were purchased from the National Institute on Aging (Bethesda, MD) at least 1 mo before the experiment, during which period they were supplied with Purina rat chow and water ad libitum. Gastric mucosal injury was induced by orogastric administration of 2 M NaCl. Groups of overnight-fasted rats (n = 5) were given by

A NUMBER OF IN VIVO and in vitro studies have demonstrated that the gastric mucosa of adult healthy animals possesses the remarkable capacity to repair promptly after a mild injury. (2, 5–8, 17, 25, 31, 32). Within minutes after injury the gastric mucosa begins to reconstitute its epithelial surface through migration of viable cells from gastric pits and glands, a process known as restitution (7, 17, 23, 25). In rats, this process is completed within about 2 h (24). Within several hours after injury, cell proliferation is increased to replace lost cells (24).

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stomach tube either 2 M NaCl (1.15 ml/100 g body wt) or an equivalent volume of water (controls) and killed 45, 90, and 240 min later. The stomach was removed, opened along the greater curvature, and rinsed thoroughly with cold normal saline. The oxyntic gland area was dissected out, and two small portions of the tissue were fixed either in 10% neutral buffered Formalin for histological evaluation or in Bouin’s fixative for immunofluorescence study. Mucosal scrapings, obtained from the rest of the oxyntic gland area (hereafter referred to as gastric mucosa), were either processed immediately (for RNA extraction) or immediately frozen in liquid nitrogen and stored at –90°C.

Mucosal histology. Formalin-fixed stomach tissues were dehydrated in graded alcohol and embedded in paraffin to yield full thickness sections of the mucosa. The paraffin-embedded tissues were serially sectioned at a thickness of 4 µm and were stained with hematoxylin and eosin or Periodic Acid Schiff stain and methylene blue for histological evaluation.

Immunofluorescent staining of EGFR. Bouin’s-fixed gastric tissue was embedded in paraffin and sectioned as stated above. Sections were collected on poly-l-lysine-coated slides. After deparaffinization and rehydration, sections were blocked with 10% chick serum, 10% porcine serum, and 0.5% BSA in PBS for 1 h at room temperature. After being washed twice with PBS, the slides were incubated overnight at 4°C with rabbit anti-EGFR antibody (polyclonal antibody, 2 µg/ml, Santa Cruz Biotechnology, Santa Cruz, CA). Rabbit γ-globulin (2 µg/ml, Sigma Chemical, St. Louis, MO) in PBS was used as a negative control. After an extensive washing in PBS, the slides were incubated with FITC-conjugated F(ab)2 fragment of anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature (20 µg/ml). After being washed in PBS three to four times (5 min each), the slides were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and sealed with nail polish. The slides were examined using a fluorescent microscope (Olympus System, model BX60, Olympus America, Lake Success, NY).

Tyrosine kinase activity. The enzyme activity was determined as previously described (19, 26). Briefly, aliquots of mucosal or parietal cell lysates were homogenized in lysis buffer [30 mM sodium phosphate, pH 7.4, 0.5% Triton X-100, 0.5% Nonidet P-40, 50 mM NaCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM Na3VO4, 10 µg/ml leupeptin, 1 µg/ml aprotinin, and 1 mM 1,10-phenanthroline] using a Dounce homogenizer. The homogenate was stirred in a mechanical rotator for 30 min at 4°C and subsequently centrifuged at 10,000 g for 10 min at 4°C. The supernatant, after dialuting with an equal volume of homogenizing buffer A (HEPES, pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM Na3VO4, 10 µg/ml leupeptin, 1 µg/ml aprotinin, and 1 mM 1,10-phenanthroline), was used as the source for tyrosine kinase. Protein content was measured using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA).

For determination of tyrosine kinase activity of EGFR, aliquots of mucosal lysate containing 200 µg protein were incubated with [γ-32P]ATP into the synthetic substrate of the polymer of L-Glu-L-Tyr (4:1) as described previously (22, 29). The results are expressed as picomoles 32P incorporated per 200 µg protein. In all immunoprecipitation studies, protein concentration was standardized among the samples.

Western blot analysis of TGF-α. For determination of the relative concentration of TGF-α in the membrane, a 30,000 g pellet was prepared from aliquots of gastric mucosal scrapings as described previously (26). Aliquots of the membrane fraction from all groups of rats containing the same amount of protein (3 mg) were incubated overnight at 4°C with 0.3 µg monoclonal antibody to TGF-α (Santa Cruz Biotechnology). The immune complexes were precipitated with protein A-Sepharose, and the immunoprecipitates were subjected to SDS-PAGE (14). After electrophoresis proteins were transferred electrophoretically onto nitrocellulose membranes (Bio-Rad) using a transfer cell (International, Mount Prospect, IL). Membranes were then incubated with blocking buffer [PBS containing 5% nonfat dry milk (Sanalac), 0.25% BSA, and 0.1% Tween 20], washed extensively with PBS containing 0.1% Tween 20, and incubated again with the same TGF-α antibodies as described previously (29). Finally, the protein bands were visualized by an enhanced chemiluminescence detection system (Amersham) according to the manufacturer’s instruction. The density of the protein bands on X-Omat films was analyzed using an image analysis system (Molecular Dynamics, Storm 860, Sunnyvale, CA). Molecular weight of the protein bands was calculated from protein markers (4–250 kDa, Novex) run concurrently.

Northern blot analysis. Steady-state mRNA levels of EGFR and TGF-α were determined using poly(A)+ RNA as described previously (29). Briefly, several aliquots of gastric mucosal scrapings from each group of rats were pooled, and total RNA was extracted by RNA-STAT solution (Tel-Test, Friendswood, TX) according to the manufacturer’s instructions. Poly(A)+ RNA was then isolated from total RNA by oligo(dT) cellulose chromatography according to the instructions provided by Gibco BRL (Gaithersburg, MD). Aliquots of poly(A)+ RNA from each group were denatured at 65°C for 15 min, subsequently mixed with an equal volume of 5× formaldehyde gel loading buffer, and size-fractionated by electrophoresis in a 1.5% agarose gel containing 2.2 M formaldehyde. After electrophoresis RNA was transferred to a Nytran membrane (Schleicher & Schuell, Keene, NH) by capillary blotting. The membrane was ultravioletly cross-linked and subsequently prehybridized in 5× SSPE buffer (0.15 mM NaCl, 11.5 mM NaH2PO4, and 1 mM EDTA) containing 50% formamide, 5× Denhardt’s reagent, 0.5% SDS, and 100 µg/ml denatured salmon sperm DNA at 55°C for 2 h.

Hybridization with 32P-labeled RNA probe was performed for 24 h at 55°C in 50% formamide, 2.5× Denhardt’s solution, 0.5% SDS, 5× SSPE, and 100 µg/ml denatured salmon sperm DNA. Antisense RNA probes from pTR1-EGFR (human) (Ambion, Austin, TX) and TGF-α (pGEM 52 f+ plasmid containing 0.623 Nco I fragment of rat TGF-α gene, generously supplied by Dr. Robert Coffey, Vanderbilt University, Nashville, TN) were prepared by transcribing 1 µg of linearized templates with SP6 RNA polymerase with the use of a commercial kit (Ambion). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal controls. This was prepared by transcribing 1 µg of pTR1-GAPDH (rat) (Ambion) linearized templates with SP6 polymerase. The probes were labeled with [α-32P]UTP according to the manufacturer’s instructions.

After hybridization, the membranes were washed twice, 15 min each time, at 50°C with 1× SSPE containing 0.5% SDS and once with 0.5× SSPE-SDS buffer. Membrane was exposed to either an X-Omat film or a Phosphor screen and was analyzed in a Phosphorimager (Molecular Dynamics). The
intensity of the EGFR mRNA signal was normalized to the GAPDH signal.

RESULTS

The extent of gastric mucosal injury in young and aged rats by hypertonic saline and the degree of epithelial restitution were evaluated by light microscopy. Immediately after injury, the gastric mucosa of both young (4 mo old) and aged (24 mo old) rats showed a complete denudation of the surface with exposed capillaries (data not shown). Basement membrane and capillaries were found to be exposed and present at the mucosal surface (Fig. 1). However, neither bleeding nor extensive ulceration was observed in any of the animals. Within 240 min of injury, significant epithelial restitution was observed in the gastric mucosa of young but not of aged rats. At this time exposed capillaries were difficult to identify in young rats (Fig. 1A), and the surface was mostly covered by attenuated epithelium (Fig. 1A, arrows). In contrast, relatively little surface epithelium was present in the gastric mucosa of aged rats (Fig. 1B, arrow), and exposed capillaries (Fig. 1B, arrow) were easily identified. This and other evidence suggest that healing was minimal in the gastric mucosa of aged rats.

Histological changes were further evaluated by examining the gastric mucosa at 45, 90, and 240 min after injury. Results are shown in Fig. 2. At 45 min after injury, the gastric mucosa from young rats showed expansion of the neck region zone and initiation of migration of foveolar cells toward the surface (Fig. 2, top, arrow). Further expansion of the neck region was observed at 90 min after injury, whereafter these changes were no longer evident (Fig. 2, bottom, arrow). Although the neck region of the gastric mucosa of noninjured aged rats (data from water-fed controls) appeared to be relatively more expanded than in their younger counterparts, further expansion of this region in aged rats was not evident until 90 min after injury. Only at 240 min after injury did we observe early migration of foveolar and parietal cells to the surface in aged rats.

To determine whether EGFR may be involved in gastric mucosal restitution, the intrinsic tyrosine kinase activity of the EGFR protein as well as expression

<table>
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<tr>
<th>Time After Injury</th>
<th>EGFR Tyrosine Kinase</th>
<th>Young rats</th>
<th>Aged rats</th>
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<tr>
<td>Control</td>
<td>1.02 ± 0.10</td>
<td>3.9 ± 0.2</td>
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<tr>
<td>45 min</td>
<td>1.90 ± 0.12*</td>
<td>3.7 ± 0.5</td>
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<td>90 min</td>
<td>2.22 ± 0.08*</td>
<td>3.1 ± 0.3</td>
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<tr>
<td>240 min</td>
<td>2.12 ± 0.05*</td>
<td>2.2 ± 0.12†</td>
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Values are means ± SE of 5–6 observations; see MATERIALS AND METHODS for units of measurement. EGFR, epidermal growth factor receptor. *P < 0.01 and †P < 0.001 compared with corresponding basal levels.
cant increase in either tyrosine kinase activity or steady-state mRNA levels of EGFR in the gastric mucosa (Table 1 and Fig. 3). In fact, at 240 min after injury, tyrosine kinase activity of EGFR in aged gastric mucosa was about 50% lower than in controls (Table 1). EGFR mRNA levels in the gastric mucosa of aged rats also remained 30–40% below the basal level throughout the reparative period (Fig. 3).

To further determine whether the age-related decrease in gastric mucosal EGFR tyrosine kinase activity during mucosal restitution could in part be related to EGFR internalization and/or degradation, immunocytochemical studies were performed. In water-fed (noninjured controls) young rats, EGFR immunoreactivity was found primarily in the surface and neck regions of gastric mucosa (Fig. 4A). There was relatively weak staining in the gland area (Fig. 4A). In these animals EGFR was primarily localized to the apical surface membrane of foveolar-type mucus cells (Fig. 4, A and D). At 45 min after injury in young rats, we observed no apparent change in EGFR expression in the neck region. However, there was an increase in staining in the glandular portion (Fig. 4B). At 240 min after injury in young rats, the relative abundance of EGFR was decreased in the neck region with a concomitant increase in the glandular portion, when compared with the water-fed controls and at 45 min after injury (Fig. 4, A and C). At this time EGFR immunoreactivity appeared mostly in intracellular vesicles (Fig. 4F, arrowhead). In contrast to young control rats (water-fed), aged control rats showed intense EGFR staining in the glandular region (Fig. 4G). EGFR staining was strongest at the apical membrane (arrow) and was faintly present as cytoplasmic vesicles within glands (Fig. 4, G and J). At 45 min after injury in aged rats, there was a marked decrease in EGFR immunoreactivity in the entire glandular compartment with decreased apical membrane staining (Fig. 4H). At this time we noted intensely bright cytoplasmic and basally oriented vesicles coincident with the decrease in apical staining (Fig. 4K, arrowhead). The number and intensity of this...
vesicular staining increased throughout the period studied (240 min, Fig. 4L).

Ligand binding to the extracellular domain of EGFR is one of the primary causes for activation of intrinsic tyrosine kinase activity of the receptor (30). Previously, we demonstrated that in young but not in aged rats TGF-α levels and EGFR tyrosine kinase activity in the gastric mucosa were significantly elevated at 30 min after injury (22). In view of the fact that TGF-α is a membrane-anchored peptide (19), we hypothesized that the age-related changes in mucosal EGFR tyrosine kinase activity during mucosal restitution could partly be due to differential accumulation of the peptide in the membrane and thereby modulating the enzyme activity through an autocrine-juxtacrine mechanism. To test this hypothesis, we examined the changes in TGF-α levels in a gastric mucosal membrane preparation from young and aged rats during the reparative period by Western blot analysis. The antibody detected two precursor forms of TGF-α with molecular masses of 14 and 15 kDa in all mucosal samples (Fig. 5). In young rats the relative concentration of both forms of TGF-α increased steadily throughout the reparative period, attaining a level of about 150% above the water-fed controls at 240 min after injury (Fig. 5). Although the basal levels of TGF-α in gastric mucosal membranes were about 40% higher in aged than in young rats (data from water-fed controls), injury in aged rats produced no significant increase in the relative concentration of either 14 or 15 kDa TGF-α when compared with the corresponding water-fed controls (Fig. 5).

To further determine whether the age-related differences in TGF-α levels in gastric mucosal membranes during mucosal restitution are under transcriptional control, mRNA levels of TGF-α were measured. Results are shown in Fig. 6. In noninjured gastric mucosa of aged rats (data from water-fed controls), in which proTGF-α levels in mucosal membranes were higher than in their younger counterparts (Fig. 5), gastric

Fig. 5. Top: Western immunoblot showing changes in relative concentration of transforming growth factor-α (TGF-α) in gastric mucosal membranes from control and 2 M NaCl-treated 4- and 24-mo-old rats. TGF-α from gastric mucosal membranes (30,000 g pellet) containing 2.4 mg protein was immunoprecipitated with TGF-α antibody, and immunoprecipitates were subjected to Western immunoblot with same antibody. Two major bands of 15 and 14 kDa were seen. Histogram (bottom) shows changes in density of both bands. Membrane fraction was prepared from pooled gastric mucosa.

Fig. 6. Top: Northern blot analysis showing changes in steady-state mRNA levels of TGF-α and GAPDH in gastric mucosa of 4- and 24-mo-old rats killed at different times after intragastric administration of 2 M NaCl. Each lane contains 2 µg of poly(A)^+ RNA. Changes in relative concentration of TGF-α mRNA, expressed as ratio of TGF-α to GAPDH, are shown in bottom.
mucosal TGF-α mRNA levels were about 50% above the young animals (Fig. 6, data from water-fed controls). In young rats, in which mucosal membrane concentration of proTGF-α increased steadily during the reparative period (Fig. 5), mRNA levels were appreciably elevated (50% above the controls) only at 240 min after injury (Fig. 6). In contrast, in aged rats, in which the levels of TGF-α in mucosal membranes were decreased after injury (Fig. 5), mucosal mRNA levels remained 40–50% below the basal levels throughout the 240-min reparative phase (Fig. 6).

**DISCUSSION**

The mucosal lining of the gastrointestinal tract, especially the stomach, is not only exposed to the deleterious effects of luminal acid but also to a variety of exogenous injurious agents, including nonsteroidal anti-inflammatory drugs and ethanol. Each of these agents either alone or in combination with others may induce injury. However, a number of in vivo and in vitro studies have demonstrated that the gastric mucosa of healthy adult animals possesses the inherent capacity to repair promptly (often within 24 h) after mild injury (2, 5–8, 17, 25, 31, 32). At least two different mechanisms are thought to participate in full repair of the damaged gastric mucosa. The rapid repair process of mucosal restitution, which begins within minutes after injury, is accomplished by cell migration and is completed within 2 h. The subsequent slower process is replacement of lost cells by cell division (24), which in rats does not peak until 18–20 h after injury (5, 24).

Although the intracellular events that regulate the reparative processes remain to be fully elucidated, recent data from this laboratory suggest a role for EGFR tyrosine kinase in regulating these processes (10, 22). The basis for this postulation comes from our observation that repeated injections of a dose of tyrphostin 51 (a tyrosine kinase inhibitor (9)) during the 24-h reparative period, which inhibits tyrosine kinase activity of EGFR, but not pp60⋅src, produces inhibition of mucosal proliferative activity as well as re-epithelialization of the mucosal surface (11). Others have demonstrated increased expression of EGFR and its ligands in the gastric mucosa during ulcer healing (26–28). Although these observations indicate a role for EGFR in gastric mucosal regeneration through cell proliferation, our recent finding that in young rats tyrosine kinase activity and expression of EGFR in the gastric mucosa are also augmented as early as 30 min after mucosal injury (22) suggests an involvement of EGFR in gastric mucosal restitution. Further support for this postulation comes from the observation that in aged rats, in which mucosal restitution is found to be attenuated (4, 5), the magnitude of stimulation of EGFR tyrosine kinase and expression of the receptor at 30 min after acute injury are considerably lower than what we noted in young rats, when compared with the corresponding water-fed controls (22). In addition, EGFR is also thought to have a role in the small intestinal restitutinal process (20). Moreover, recent evidence suggests a role for EGFR in regulating polyamine-induced stimulation of cytoskeleton reorganization in IEC cells after injury (16). These and other relevant observations led us to suggest that decreased activation of the EGFR signaling pathway in aged gastric mucosa after injury may contribute to attenuation of mucosal restitution (10, 22). Our current data support this contention. We have observed that in young but not in aged rats gastric mucosal EGFR tyrosine kinase activity is greatly stimulated within 45 min after injury and remains elevated throughout the 240-min reparative period. This early rise in mucosal EGFR tyrosine kinase activity after injury in young rats is not associated with increased transcription of EGFR.

Although a number of factors, including expression of EGFR, are known to modulate the intrinsic tyrosine kinase activity of the receptor, ligand binding to its extracellular domain is considered to be one of the primary causes for activation of the enzyme activity (3, 30). Although both EGF and TGF-α bind to EGFR with equal potency, the observation that in rats levels of TGF-α but not EGFR are substantially elevated in the gastric mucosa and gastric juice shortly after acute injury (21) suggests a role for endogenous TGF-α in regulating EGFR tyrosine kinase activity during the early reparative phase through an autocrine-paracrine mechanism. However, TGF-α is a membrane-anchored peptide (19). Results from cell surface and biochemical characterization studies have demonstrated that the presence of the transmembrane TGF-α is a normal consequence of TGF-α synthesis, and in most cases the peptide is present on the cell surface in its precursor form (1, 15, 18). Moreover, it has been demonstrated that the transmembrane TGF-α precursor form activates EGFR (1). Taken together, these observations suggest that the membrane-bound TGF-α may regulate EGFR tyrosine kinase activity through an autocrine-paracrine mechanism. In support of this postulation we have observed that the age-related rise in gastric mucosal EGFR tyrosine kinase activity is associated with a concomitant increase in the relative concentration of 18 and 14 kDa precursor forms of TGF-α in mucosal membranes (29). The same mechanism may also be responsible for the age-related changes in mucosal EGFR tyrosine kinase activity during the current reparative period. We have observed that in young rats, the relative concentration of 14 and 15 kDa precursor forms TGF-α in gastric mucosal membrane fractions increased steadily during the 240-min reparative period. In contrast, in aged rats, with diminished gastric mucosal restitutinal ability (4, 5), the relative concentration of both forms of TGF-α in mucosal membranes remained below the water-fed controls throughout the reparative period. The fact that changes in TGF-α levels in mucosal membranes of aged rats are also accompanied by parallel alterations in mucosal EGFR tyrosine kinase further suggests that membrane-bound form(s) of TGF-α may have a role in modulating EGFR tyrosine kinase activity during mucosal repair. Therefore, decreased activation of the EGFR signaling pathway in aged gastric mucosa after injury as a result of diminished accumulation of proTGF-α can be consid-
er a ne explanation for the attenuation of mucosal restitution in these animals.

The decreased activation of EGFR tyrosine kinase in aged rats after injury could also be the result of increased internalization and/or degradation of the receptor. Support for this interpretation comes from the observation that at 45 min after mucosal injury EGFR immunoreactivity associated with intracellular particles in gastric mucosal cells is found to be higher in aged than in young rats. Although the reasons for this are not fully understood, it is plausible that increased binding of TGF-α to EGFR in aged rats as a result of higher basal levels of both TGF-α and EGFR in the gastric mucosa (data from water-fed controls) may induce rapid internalization of the receptor-ligand complex after the injury-induced stimulus. Recently, we have also observed that aging enhances sensitivity of the gastric mucosa to both EGF and TGF-α so that low doses activate mucosal EGFR, whereas large doses inhibit this activation (12). Therefore, the age-related decline in mucosal repair could be due to decreased activation of EGFR tyrosine kinase resulting from increased sensitivity of the receptor to it, in that doses of the ligands needed to activate EGFR tyrosine kinase in the gastric mucosa of aged rats are lower than those required for young animals. Further experiments are undoubtedly necessary to fully elucidate the role of EGFR in gastric mucosal reparative processes during aging.

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