Janus kinase 3 (Jak3) is a non-receptor tyrosine kinase known to be expressed in hematopoietic cells. Studies of whole organ homogenates show that Jak3 is also expressed in the intestines of both human and mice. However, neither its expression nor its function has been defined in intestinal epithelial enterocytes. The present study demonstrates that functional Jak3 is expressed in human intestinal enterocytes HT-29 CI-19A and Caco-2 and plays an essential role in the intestinal epithelial wound repair process in response to interleukin 2 (IL-2). Exogenous IL-2 enhanced the wound repair of intestinal enterocytes in a dose-dependent manner. Activation by IL-2 led to rapid tyrosine phosphorylation and redistribution of Jak3. IL-2-stimulated redistribution of Jak3 was inhibited by the Jak3-specific inhibitor WHI-P131. IL-2 also induced Jak3-dependent redistribution of the actin cytoskeleton in migrating cells. In these cells Jak3 interacted with the intestinal and renal epithelial cell-specific cytoskeletal protein villin in an IL-2-dependent manner. Inhibition of Jak3 activation resulted in loss of tyrosine phosphorylation of villin and a significant decrease in wound repair of the intestinal epithelial cells. Previously, we had shown that tyrosine phosphorylation of villin is important for cytoskeletal remodeling and cell migration. The present study demonstrates a novel pathway in intestinal enterocytes in which IL-2 enhances intestinal wound repair through mechanisms involving Jak3 and its interactions with villin.

Epithelial cells in the gastrointestinal mucosa play an important role in defining the physical barrier between the host and the external environment. This protection by intestinal epithelial cells (IEC) \(^2\) is maintained by a highly dynamic and continuous cross-talk between the IEC and the immune cells of the gastrointestinal tract. Intraepithelial lymphocytes and immune cells of the lamina propria express cytokines such as interleukin 1 (IL-1), IL-2, IL-6, tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), and TNF-\(\beta\). Many of these cytokines are known to stimulate proliferation and differentiation of IEC cells (1–3). However, the role of these immune cell-derived cytokines in the repair of gastrointestinal mucosa has not been well defined. Functional IL-2 receptors have been identified in both rat and human intestinal epithelial cells (4, 5), and IL-2 knock-out mice develop ulcerative colitis (6). In addition, IL-2 expression is reduced in human colitis patients (7).

The immediate response to injury of the gastrointestinal epithelium is directed primarily toward restoring the disturbed epithelial continuity through cell spreading and migration. Previous studies have shown that exogenous IL-2 enhances the migration of undifferentiated rat IEC (3), but the mechanisms have not been elucidated. Janus kinase 3 (Jak3) is a non-receptor tyrosine kinase that interacts with receptors for IL-2, IL-5, IL-7, IL-9, and IL-15 through the common \(\gamma\)-chain (8). Northern analysis studies show that Jak3 is expressed in different organs including the small intestine and kidney of both humans and mice (8, 9). Previous studies with IL-2R\(\gamma\)- and Jak3-null mice showed that both of these strains developed spontaneous inflammatory bowel disease symptoms including damaged intestinal mucosa (10). This suggests that both proteins may be important for intestinal wound repair, but the role of Jak3 has not been investigated previously in intestinal epithelial repair mechanisms.

Villin is an actin-binding protein expressed in specific epithelial cells including those of the intestines. It can nucleate, cap, sever, and bundle actin filaments. Previously we have shown that the actin modifying activity of villin can be regulated by either its binding to different ligands (e.g. \(Ca^{2+}\), PIP2) or through tyrosine phosphorylation (11–13). We have also shown that tyrosine phosphorylation of villin is necessary for its role in cell migration (14). Also the migratory capacity of IEC was shown to be reduced in villin knock-out mice during an experimental colitis condition (15). In this report, we demonstrate that intestinal epithelial cells express functionally specific Jak3, a potent non-receptor tyrosine kinase whose biological functions have been presumed to be largely limited to lymphocyte and macrophage populations (8), and propose a mechanism through which activated Jak3 and villin regulate intestinal epithelial wound repair.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and IL-2 Treatment**—HT-29 CI-19A and Caco-2 are human-derived differentiated intestinal epithelial cell lines. These were maintained in DMEM containing 10% fetal calf serum (Invitrogen). For IL-2 treatments, confluent monolayers of cells were kept in DMEM containing 0.1% fetal calf serum for 17 h before replacement with fresh media containing human recombinant IL-2 (Sigma).

**Immunoprecipitations (IP) and Immunoblotting**—For IP confluent Caco-2 and HT-29 CI-19A cells were lysed using lysis buffer containing 20 mM Tris, pH 7.4, 150 mM NaCl, 10% glycerol,
IL-2 stimulates IEC migration and in human colitis patients (7), we investigated whether found to be important in experimental models of colitis (6) immunoprecipitated with an anti-phosphotyrosine antibody IEC with IL-2 for different periods of time, cell lysates were determined the activation of Jak3, an IL-2 receptor-interacting molecular basis of IL-2-induced wound closure in IEC, we expressed in intestinal epithelial cells (18). To understand the freshly, and there is only one report to date showing that Jak3 is intestinal epithelial cells is mediated by Jak3 activation. closure was specifically diminished by both inhibitors to the either the pan-Jak inhibitor AG-490 or the Jak3-specific in vitro—An kinase assay was done as reported in vivo compartmentalization. However, treatment with IL-2 for 15 min led to redistribution of Jak3 to the cell margin in subconfluent cells (Fig. 2C) with higher concentrations of Jak3 localized in regions of the cells where there were intercellular spaces (indicated by arrows). These regions typically contain lamellipodial and filopodial extensions as the cell spreads (enlarged view of single cell in the lower panel of Fig. 2C). We chose the 15-min time point because the maximum phosphorylation of Jak3 following IL-2 treatment occurs at that time. The redistribution of Jak3 to the cell margin was inhibited by the Jak3 inhibitor WHI-P131, as shown in Fig. 2D, and the enlarged view of a single cell in the corresponding lower panel. These results demonstrate that IL-2-induced activation of Jak3 is necessary for its recruitment at the cell margin.

IL-2 Promotes Redistribution of Jak3 in IEC—In immunocytes Jak3 interacts with the \( \gamma \)-subunit of the activated IL-2 receptors and localizes to the plasma membrane (19). To determine whether IL-2 regulates the distribution of Jak3 in IEC, HT-29 CI-19A cells were treated with IL-2, and the distribution of Jak3 was analyzed by IFM using a Jak3-specific monoclonal antibody. As shown in Fig. 2B (with an enlarged view of a single cell in the lower panel) the distribution of Jak3 in serum-starved HT-29 CI-19A cells (Control) is cytoplasmic without clear compartmentalization. IL-2 stimulation of Jak3 to the cell margin in subconfluent cells (Fig. 2C) with higher concentrations of Jak3 localized in regions of the cells where there were intercellular spaces (indicated by arrows). These regions typically contain lamellipodial and filopodial extensions as the cell spreads (enlarged view of single cell in the lower panel of Fig. 2C). We chose the 15-min time point because the maximum phosphorylation of Jak3 following IL-2 treatment occurs at that time. The redistribution of Jak3 to the cell margin was inhibited by the Jak3 inhibitor WHI-P131, as shown in Fig. 2D, and the enlarged view of a single cell in the corresponding lower panel. These results demonstrate that IL-2-induced activation of Jak3 is necessary for its recruitment at the cell margin.

IL-2 Promotes Wound Closure in IECs—Because IL-2 was found to be important in experimental models of colitis (6) and in human colitis patients (7), we investigated whether IL-2 stimulates IEC migration in vitro using a scratch wound assay. As shown in Fig. 1 and supplemental Fig. S1 recombinant human IL-2 stimulated wound closure in the differentiated HT-29 CI-19A and Caco2 (supplemental Fig S2, A and B) cells in a dose-dependent manner. Treatment of IECs with IL-2 led to more than a 2-fold increase in wound closure of both of these cell lines. To determine whether IL-2-stimulated cell migration was mediated by activation of Jak in general and Jak3 in particular, the cells were treated with either the pan-Jak inhibitor AG-490 or the Jak3-specific inhibitor WHI-P131. The IL-2-stimulated increase in wound closure was specifically diminished by both inhibitors to the same extent, suggesting that IL-2-induced wound closure in intestinal epithelial cells is mediated by Jak3 activation.

IL-2 Induces the Activation of Jak3—The functional role of Jak3 in intestinal epithelial cells has not been identified previously, and there is only one report to date showing that Jak3 is expressed in intestinal epithelial cells (18). To understand the molecular basis of IL-2-induced wound closure in IEC, we determined the activation of Jak3, an IL-2 receptor-interacting kinase, in response to IL-2 treatment. Following treatment of IEC with IL-2 for different periods of time, cell lysates were immunoprecipitated with an anti-phosphotyrosine antibody (pY20) and then were immunoblotted with a Jak3-specific antibody. As shown in Fig. 2A (upper panel), IL-2 induced an increase in tyrosine phosphorylation of Jak3 in both HT-29 CI-19A and Caco2 (supplemental Fig S3) cells. Although phosphorylation increased rapidly and then decreased by 120 min, phosphorylation of Jak3 was sustained at a high level for at least 60 min. These results demonstrate that activation of Jak3 is not restricted to immunocytes but also occurs in IEC.

IL-2 Stimulates Jak3 Interactions with Villin—Given that villin plays a significant role in cytoskeletal remodeling and cell migration in IEC (14), we tested the hypothesis that IL-2-stimulated cell migration in intestinal enterocytes was mediated through activation of villin. Following IL-2 treatment of HT-29 CI-19A cells, cell lysates were immunoprecipitated with pY20 and immunoblotted with an antibody against villin. As shown in Fig. 3A (upper panel), treatment of IEC with IL-2 led to a rapid increase in tyrosine phosphorylation of villin for up to 15 min with a subsequent decrease by 60 min. To test the hypothesis that Jak3 is responsible for the tyrosine phosphorylation of villin in IL-2-stimulated intestinal enterocytes, the cells were treated with WHI-P131, and the tyrosine phosphorylation of villin was assessed. Fig. 3B shows that the tyrosine phosphorylation of villin stimulated by 15 min of IL-2 treatment was completely blocked by the Jak3 inhibitor WHI-P131. To determine whether Jak3 associates with villin, we immunoprecipitated cell lysates from IL-2-treated or untreated cells with mouse IgG (as a negative control), Jak3 (as a positive control), or villin-specific antibodies and immunoblotted with the Jak3-specific antibody. As shown in Fig. 3C, IL-2 treatment stimulated association of Jak3 with villin, and this association was blocked by WHI-P131. The association of Jak3 with villin was also confirmed by IFM (supplemental Fig S4). Supplemental Fig S4 shows that Jak3 did not co-localize with villin in control cells (A), but treatment with IL-2 led Jak3 to co-localize with villin at the cell periphery and filopodial extension (B). Co-localization was not apparent in cells treated with IL-2 in the presence of...
Jak3 inhibitor WHI-P131. To determine whether Jak3 directly phosphorylates villin, we performed a cell-free kinase assay using bacterially expressed recombinant villin and Jak3 kinase. The autoradiogram of SDS-PAGE-separated reaction mixtures shows that Jak3 directly phosphorylates villin in the presence of radioactive ATP (Fig. 3D, upper panel). For these experiments villin alone was used as a negative control to show nonspecific radioactivity associated with villin and villin + Src was taken as a positive control, because Src is a known kinase for villin (14). The input control for equal amounts of recombinant villin is shown in Fig. 3D, lower panel. These results suggest that villin is a direct cellular substrate of Jak3.

**IL-2 Promotes Jak3-dependent Actin Redistribution in Migrating Enterocytes**—Given that IL-2 stimulates phosphorylation of villin through activation of Jak3, we investigated whether IL-2 affects the distribution of actin in migrating enterocytes and whether this distribution is dependent on Jak3 activation. HT-29 CL-19A cells were first wounded and then treated with IL-2 for 15 min in the presence or absence of the Jak3 inhibitor WHI-P131. The cells were fixed and stained with rhodamine-conjugated phalloidin, and planar distributions of F-actin were examined using confocal microscopy. Fig. 3E shows a uniform distribution of F-actin in planes at different distances from the substratum in control cells, whereas cells treated with IL-2 for 15 min exhibit a loss of actin filaments from the basal planes (Fig. 3F). Inhibition of Jak3 with WHI-P131 partially prevented the loss of F-actin from the basal planes (Fig. 3G). WHI-P131 alone had no effect on actin redistribution (supplemental Fig. S5). To quantify these results, we measured the average fluorescence per unit area of the cells as a function of the distance from the substratum. Fig. 3H summarizes these results from seven different fields from three independent experiments for each condition. Although substantial levels of F-actin were measured in the first 5-μm distance from the substratum in control cells, very little F-actin was measured in these planes in IL-2-treated cells. However, treatment with WHI-P131 partially blocked the F-actin redistribution caused by IL-2. This may be due to the presence of other actin-remodeling proteins activated by IL-2.

**DISCUSSION**

IL-2 is secreted by lymphocytes and plays a major role in stimulating proliferation of mucosal lymphocytes, natural killer cells, and macrophages (20). Until recently, IL-2 was thought to be restricted to regulating the growth and differentiation of immunocytes during inflammatory response (21, 22), but there is increasing evidence that IL-2 is important for the maintenance and repair of the gut mucosa. Functional IL-2 receptors have been identified in several intestinal cell lines, in rat small intestinal enterocytes, and in human colonocytes, implying a physiological role for IL-2 in modulating epithelial function (4, 5). Also, human colitis patients show substantial reduction in the expression of IL-2 (7). Moreover, disruption of the IL-2 gene leads to prolonged intestinal inflammation, severe ulcerative colitis, and death in knock-out mice, suggesting that IL-2 is important for the control of inflammation in the intestine (6).

We hypothesized that IL-2 helps to minimize intestinal inflammation by enhancing the wound repair of IEC. To test this hypothesis we studied intestinal epithelial wound repair in vitro using post-confluent differentiated IECs treated with recombinant human IL-2. Our results show that IL-2 increased IEC wound repair in a dose-dependent manner. However, at higher concentrations of IL-2 the mucosal wound repair was inde-
pendent of IL-2 concentrations. We speculate that immune cell production of IL-2 is tightly regulated in the normal intestine but is up-regulated following injury to facilitate wound repair. IL-2 has been shown to enhance wound repair of undifferentiated IEC, but the mechanism has not been determined (3). We investigated the mechanism through which IL-2 regulates intestinal epithelial wound repair.

Our results demonstrate that IL-2 induces cell migration in

FIGURE 3. A–C, IL-2 stimulates the interactions of Jak3 with villin. A, time course of IL-2-stimulated tyrosine phosphorylation of villin. Equal amounts of villin protein (input control, lower panel) from cells treated with IL-2 (50 units/ml) for the indicated times were immunoprecipitated with pY20 and immunoblotted (IB) with an antibody against villin (upper panel) using procedures similar to those described in Fig. 2. B, inhibition of Jak3 blocks the tyrosine phosphorylation of villin. Procedures similar to those described in A were followed, except the cells were treated with IL-2 in the presence or absence of the Jak3 inhibitor WHI-P131. C, Jak3 interacts with villin in an IL-2-dependent manner. Cells were treated with or without IL-2 for 1 h and subjected to immunoprecipitation as mentioned under “Experimental Procedures” using IgG, Jak3-, and villin-specific antibodies. The SDS-PAGE-separated protein complex was immunoblotted with a Jak3-specific antibody. The lanes shown are from the same blot (unrelated lanes were removed for clarity).

D, Jak3 directly phosphorylates recombinant villin in vitro. An in vitro kinase assay was done as reported previously (16). E–G, IL-2-stimulated Jak3 activation leads to disassembly of actin filaments from the basal plane of migrating cells. E, planar distribution of F-actin in HT-29 Cl-19A cells at the wound edge of control cells; F, cells treated with IL-2; G, cells treated with IL-2 + WHI-P131. The lower images in each panel are the corresponding bright field images. Arrows in E–G indicate the wound edge. H, normalized F-actin intensity for each plane as a function of the distance from the substratum. These results were the average of seven different fields from three independent experiments. The top left corner of each panel in E–G denotes the basal plane, and the number below each plane denotes the height (in μm) of that plane from the basal plane. Procedures similar to those described in Fig. 2, B–D, were followed, except rhodamine-conjugated phalloidin was used to stain F-actin in the paraformaldehyde-fixed cells. For planar distribution of actin, the first plane of the cells was determined using bright field images through the adjustment of the focal plane using the software LSM Pascal (Zeiss, Jena, Germany) to the point where no images were visible and above which the boundary of cells was visible with a 1 μm change of the focal plane. The subsequent 19 planes were taken using the first plane as a reference. Images were stacked and processed using NIH ImageJ software.
the intestinal enterocytes through activation of Jak3. Janus kinases play an essential role in cytokine signaling and have been reported to regulate plasma membrane expression of their cognate receptors (19). For example, overexpression of Jak3 in HeLa cells enhanced accumulation of the γ-chain (γc) of the IL-2 receptor at the plasma membrane. Moreover, membrane expression of γc was necessary for the membrane localization of Jak3, where it co-localized with the γc of IL-2 receptors (19).

We demonstrated that IL-2 stimulated wound repair in intestinal enterocytes through time-dependent tyrosine phosphorylation of Jak3 (Fig. 2A), which led to the redistribution of Jak3 to the plasma membrane (Fig. 2C). Jak3 appeared to localize into subcellular structures at the edge of cells, which may be important in the wound closure process. We speculate that IL-2-stimulated activation of Jak3 led to Jak3 recruitment at the plasma membrane through the γc of IL-2 receptors.

We demonstrated a novel mechanism through which IL-2-stimulated wound closure in intestinal enterocytes involves a time-dependent tyrosine phosphorylation of the intestinal cytoskeletal component villin. Furthermore, Jak3 interacts directly with villin, and inhibition of Jak3 prevents IL-2-induced villin phosphorylation. Also, Jak3 directly phosphorylates recombinant villin in a cell free assay. Moreover tyrosine phosphorylation of Jak3 in cells remained elevated after 6 h (~10-fold higher compared with the initial level), whereas tyrosine phosphorylation of villin returned to the initial level by 1 h. To our knowledge this is the first report showing direct interactions of Jak3 with any actin-binding protein and its functional significance. We speculate that Jak3 and villin may coordinate the initiation of the wound repair process, and this may be followed by alternative signaling pathways during long-term repair mechanisms. Src kinase was previously reported to stimulate tyrosine phosphorylation of villin during epithelial growth factor-induced wound repair of intestinal epithelial cells (14). Our studies demonstrate an alternative pathway for the tyrosine phosphorylation of villin during intestinal wound repair.

Rearrangement of the actin cytoskeleton is essential for cell migration during wound repair, and our results demonstrate for the first time that IL-2 stimulates planar redistribution of F-actin in migrating cells (Fig. 3E). IL-2 treatment led to the loss of F-actin from the basal plane of the migrating enterocytes within 15 min. This loss of F-actin may facilitate two processes important for cell migration. First, the severing of F-actin filaments at the leading edge of cells generates new actin nuclei for migration during wound repair, and our results demonstrate an alternative pathway for the tyrosine phosphorylation of villin during intestinal wound repair.

In summary, our results demonstrate the molecular mechanism of the early steps by which IL-2 enhances mucosal wound repair in an in vitro wound closure model. These findings support the hypothesis that IL-2 plays an important role in mucosal wound repair in response to infections and inflammation. This is of particular importance given the locations in the intestine where various constituents of the lamina propria and intraepithelial lymphocytes express IL-2. During various forms of intestinal injury the immune system may be activated, resulting in an elevated concentration of IL-2 in the vicinity of IEC (23, 24). Thus, we hypothesize that IL-2 produced by different constituents of the intestine may promote mucosal wound repair mediated by Jak3 activation and cytoskeletal remodeling involving tyrosine phosphorylated villin.

Acknowledgments—Caco-2 and HT-29 Cf-19A cells were provided by Dr. G. Tsigi and Dr. A. P. Naren, respectively (both from the Dept. of Physiology, University of Tennessee Health Science Center, Memphis). Recombinant villin was a gift from Dr. R. L. Rimm (Dept. of Pathology, Yale University School of Medicine).

REFERENCES

1. Sansonetti, P. J. (2004) Nat. Rev. Immunol. 4, 953–964

2. Rescigno, M., and Chieppa, M. (2005) Nat. Med. 11, 254–255

3. Dignass, A. U., and Podolsky, D. K. (1996) Exp. Cell Res. 225, 422–429

4. Ciacci, C., Mahida, Y. R., Dignass, A., Koizumi, M., and Podolsky, D. K. (1993) J. Clin. Investig. 92, 527–532

5. Reinecker, H. C., and Podolsky, D. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8353–8357

6. Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C., and Horak, I. (1993) Cell 75, 253–261

7. Melgar, S., Yeung, M. M., Bas, A., Forsberg, G., Suhr, O., Oberg, A., Hammarström, S., Danielsson, A., and Hammarström, M. L. (2003) Clin. Exp. Immunol. 134, 127–137

8. Safford, M. G., Levenstein, M., Tsifrina, E., Amin, S., Hawkins, A. L., Grif- fin, C. A., Civin, C. I., and Small, D. (1997) Exp. Hematol. 25, 374–386

9. Takahashi, T., and Shirasawa, T. (1994) FEBS Lett. 342, 124–128

10. Murata, Y., Yamashita, A., Saiito, T., Sugamura, K., and Hamuro, J. (2002) Int. Immunol. 14, 627–636

11. Kumar, N., Tomar, A., Parrill, A. L., and Khurana, S. (2004) J. Biol. Chem. 279, 45036–45046

12. Kumar, N., Zhao, P., Tomar, A., Galea, C. A., and Khurana, S. (2004) J. Biol. Chem. 279, 3096–3110

13. Kumar, N., and Khurana, S. (2004) J. Biol. Chem. 279, 24915–24918

14. Tomar, A., Wang, Y., Kumar, N., George, S., Ceacareanu, B., Hassid, A., Chapman, K. E., Aryal, A. M., Waters, C. M., and Khurana, S. (2004) Mol. Biol. Cell 15, 4807–4817

15. Ferrary, E., Cohen-Tannoudji, M., Pehau-Arnaudet, G., Lapillonne, A., Athman, R., Ruiz, T., Boulouha, L., El Marjou, F., Loye, A., Fontaine, J. J., Antony, C., Babinet, C., Louvard, D., Jaisser, F., and Robine, S. (1999) J. Cell Biol. 146, 819–830

16. Wang, R., Griffin, P. R., Small, E. C., and Thompson, J. E. (2003) Am. J. Pathol. 167, 969–980

17. Hofmann, S. R., Lam, A. Q., Frank, S., Zhou, Y. J., Ramos, H. L., Kanno, Y., Fung, V., Beers, C., Richardson, J., Schoenborn, M. A., Ahdieh, M., et al. (1994) Science 264, 965–968

ACCELERATED PUBLICATION: Jak3 Regulates Intestinal Wound Repair

OCTOBER 19, 2007 • VOLUME 282 • NUMBER 42

JOURNAL OF BIOLOGICAL CHEMISTRY

30345