Mitogen-activated protein kinases (MAPK) are among the major widespread transduction pathways in humans. They are involved in several inflammatory disorders, including the pathogenesis of inflammatory bowel disease (IBD). A recent paper showed that activated MAPK are upregulated on endothelium and fibroblasts from intestinal biopsies of active IBD patients. In vitro experiments demonstrated that MAPK activation on intestinal endothelial cells and fibroblasts are responsible for the production of certain chemokines, increased leukocyte adhesion and transmigration. Specific local inhibition of MAPK activity on endothelial cells and fibroblasts may provide a new mechanism to control mucosal inflammation and leukocyte recruitment into the intestine of active IBD patients.

Mitogen-activated protein kinases (MAPK) belong to major and widespread signal transduction pathways, involved in cell cycle, apoptosis, cancer progression, development and differentiation as well as in inflammation. They can be activated by a wide variety of stimuli, including hormones, growth factors, inflammatory cytokines and environmental factors such as radiation, osmotic stress and ischemic injury. Three major groups of distinctly regulated MAPK cascades are known in humans which lead to altered gene expression: ERK1/2 (known also as p42/p44), JNK, and p38 MAP kinase. All MAPK are activated upon phosphorylation by other kinases (MAPK kinases, MKK), which are in turn activated by other kinases (MAPK kinase kinases, MKKK) (Fig. 1). In particular, ERK1/2 is activated by MKK2, JNK by MKK4 and MKK7 while p38 MAP kinases are activated by MKK3, MKK4 and MKK6.1,2 The activation consists in the phosphorylation of both threonine and tyrosine residues. Once activated MAPK phosphorylate the target substrates on serine or threonine residues only if the amino acid residues are followed by proline. MAPK target substrates including transcription factors, other kinases or proteins such as cytoskeletal proteins.

Other proteins, such as protein phosphatases (MAP kinase phosphatases, MKP), are responsible for downregulating MAPK activity by removing the phosphoric residue from active MAPK. Although MAPK has been described to be involved in different processes its involvement in inflammation, in particular the maintenance of chronic inflammation has become an interesting field of study.

All three MAPK families were in fact described in different experimental systems to actively participate in the upregulation of pro-inflammatory genes in response to different stimuli. For instance p38 MAPK positively regulates expression of many genes involved in inflammation, such as those coding for TNFα, IL-1, IL-6, IL-8, cyclooxygenase-2, and collagenase-1, -3 in different cell types, especially monocytes and macrophages.3-5 Based on these findings several MAPK inhibitors were characterized as potent anti-inflammatory drugs. Pyridinyl imidazole compounds, exemplified by SB 203580, originally developed as inhibitors of inflammatory cytokine synthesis,10,11 are selective inhibitors of p38 MAPK (isoforms “a” and “h”), blocking the catalytic activity of the kinase by competitive binding in the ATP pocket.12

A potent JNK/p38 MAPK inhibitor, CNI-1493 (a synthetic guanylhydrazone) was used in a human trial and showed a clinical benefit and mucosal healing in patients with severe Crohn disease.13-15 Despite experimental and clinical data the exact mechanisms of action of MAPK and their relevance in diseases such as inflammatory bowel disease (IBD) is not clear.

Most of the information available regarding the role of MAPKs in IBD is their involvement in leukocyte functions.
Waetzig et al. demonstrated that the activated form of p38, the JNKs and ERK (in this paper indicated as p42/44) are upregulated in patients with IBD while their inactive forms are decreased particularly in lamina propria macrophages and neutrophils. Mitsuyama et al. identified the nucleus of epithelial and lamina propria mononuclear cells as the major source of activated MAPK in patients with IBD. However, in IBD an important pathogenic role in chronic inflammation is played also by non-immune cells, such as endothelial cells and fibroblasts. These cells have been described to be particularly active in responding to pro-inflammatory stimuli, such as TNFα, one of the main pro-inflammatory cytokines involved in IBD pathogenesis. The TNFα dependent activation of these non immune cells is responsible for the production of pro-inflammatory mediators, chemokines and the upregulation of important adhesion molecules.

The role of MAPK, in particular p38, ERK and JNK, in the pro-inflammatory response of intestinal fibroblasts and endothelial cells has been recently described by our group. Phosphorylated MAPK levels are highly expressed in active IBD intestinal mucosa compared to healthy controls. Histological studies showed that activated MAPK are present on different mucosal cell types, in particular on endothelial cells and fibroblasts. These findings confirm that MAPK activation is associated with chronic inflammation of the gut mucosa, as it occurs in IBD. Furthermore our data showed that MAPK activation may play a crucial role in IBD pathogenesis in particular in maintaining chronic inflammation, via new mechanisms involving new characters of mucosal immunity: non immune cells.

MAPK activation, normally described in immune cell activation including monocytes, neutrophils and macrophages, is now also shown in non-immune cells of the intestinal mucosa, in particular in fibroblasts and endothelial cells.

These findings suggest that MAPK acts through different cell populations to sustain inflammation, in a broader and much more complex way, than previously described.

To better define the involvement of MAPK in intestinal non-immune cell activation, we used an in vitro system, with primary colonic cells, human intestinal fibroblasts (HIF) and human intestinal mucosal endothelial cells (HIMEC).

TNFα, chosen as the pivotal pro-inflammatory stimulus, was able to induce the activation of all three MAPK studied, in both HIF and HIMEC. The activation of MAPK was highest between 5 minutes and 2 hours following TNFα stimulation but was still visible after 24 hours of stimulation, especially for p38 and ERK. The TNFα dependent activation of MAPK in non-immune cells observed in vitro corresponds with the findings showed by histology on human colonic samples, where active IBD intestinal mucosa, rich in TNFα content, showed greater expression of the activated forms of all three MAPK in fibroblasts and endothelium compared to controls.

The activation of MAPK was accompanied by the upregulation of important cytokines and chemokines involved in leukocyte recruitment, such as IL8, MCP-1, RANTES, SDF-1 and IP-10,
by both HIMEC and HIF. Along with the increased secretion of soluble mediators, TNFα induced MAPK activation was also followed by ICAM-1 and VCAM-1 upregulation by HIMEC as shown by flow cytometric analysis. These molecules have a crucial role in mediating the adhesion of leukocytes to endothelial cells.

The cause-effect relationship between the observations reported and MAPK activation was demonstrated using specific MAPK inhibitors. In particular SB203580 was used to inhibit p38, PD98059 to inhibit ERK and SP600125 to inhibit JNK specific activation and function. (Fig. 1) Indeed, specific blockade of p38 activation by SB203580 and ERK activation by PD98059 reduced TNFα induced production of IL8 and MCP-1 by both HIF and HIMEC. The same treatment was able to selectively reduce the TNFα dependent upregulation of ICAM-1 and VCAM-1 by HIMEC.

These findings suggest that MAPK activation is responsible for an increased production of inflammatory soluble mediators as well as upregulation of surface adhesion molecules by non immune cells. These events may have important in vivo implications, such as increased leukocyte recruitment into inflamed tissue. While leukocyte recruitment itself may help with the clearance of microbial agents in the course of acute inflammation, it can be however detrimental in the course of chronic inflammation where the immune response builds up towards self antigens. To clarify the functional effects of these findings, we performed additional in vitro experiments, mimicking the conditions in vivo.

Briefly, in order to prove that MAPK activation in non immune cells is associated with increased leukocyte recruitment in the inflamed mucosa (as it occurs in IBD), we created an in vitro model which enabled us to explore leukocyte recruitment. As it has been clearly established, leukocyte recruitment is a key pathogenic event which occurs in vivo through different phases: adhesion of leukocytes to endothelial cells, transmigration through the endothelium and chemotaxis towards the inflamed tissue containing high concentrations of inflammatory chemokines and cytokines. Taking into consideration the different phases of leukocyte recruitment we began our study by first exploring leukocyte adhesion to HIMEC. As expected HIMEC exposed to TNFα stimulation adhered a large number of leukocytes on their surface. When HIMEC were incubated with specific p38 and ERK inhibitors a clear decrease in leukocyte adhesion was observed, suggesting that MAPK play a pivotal role in this phenomenon.

We then used another experimental procedure to explore leukocyte chemotaxis induced by HIF through a HIMEC monolayer. Chemotaxis is the next phase in leukocyte recruitment following adhesion which allows immune cells to invade the peripheral tissue.

In the experimental system used, chemotaxis is directed towards chemochines produced by HIF after TNFα stimulation. As expected a significant leukocyte chemotaxis through the HIMEC monolayers was observed in the presence of supernatants from TNFα treated HIF. When HIF were incubated with p38 and ERK inhibitors and simultaneously stimulated with TNFα a clear decrease in leukocyte transmigration was observed, suggesting that p38 and ERK activation are crucially involved in TNFα dependent leukocyte chemotaxis towards inflamed fibroblasts.

Overall the above reported results support the role of MAPK in the response of two types of non-immune cells of the gut mucosa to inflammatory stimuli. These observations are particularly relevant as they come from human samples, both mucosal biopsies and primary cells.

Among the explored MAPK, although all three seem to be activated by inflammatory stimuli in non immune cells, only p38 and ERK showed a clear role in TNFα induced leukocyte adhesion and chemotaxis by HIF and HIMEC, suggesting that the phenomenon observed are specific.

Furthermore the importance of MAPK in the inflammatory process as a result of activation of non-immune cells was highlighted; this new mechanism of action of MAPK in intestinal inflammation can be considered an important potential novel therapeutic target.

The main and best characterized anti MAPK drug is the anti p38 MAPK. Its efficacy was shown in preclinical studies20,21 and even in clinical trials22 as indicated also above.22 Most of these trials showed several side effects mainly related to liver and CNS toxicity, which led to the suspended use of these drugs. The most plausible reason of toxicity is that because p38 is a molecule involved in many processes, the systemic use of blocking drugs may exert undesirable effects, especially in organs not directly involved in chronic inflammation, such as the liver and the brain in the course of IBD. Others report that targeting p38 activation systemically leads to inhibition of the feedback control loops which suppress the activities of ‘upstream’ MAPK kinase kinases, implicated in the activation of other pro-inflammatory pathways, such as activation of JNK. Further studies, however, are necessary to clarify these mechanisms.

Following the perspective of the proposed study, one can argue that specifically targeting local characters of intestinal inflammation, such as fibroblasts and endothelial cells, may result in a more efficacious and well tolerated therapeutic strategy compared to a systemic approach. The local inhibition of MAPK activation in mucosal fibroblasts and endothelium can provide the basis to inhibit leukocyte-endothelial interactions at the molecular level, inhibiting the aberrant homing of leukocytes to the gut in patients affected by IBD. This goal can be obtained also with the use of drug carriers able to directly deliver the drug to the inflamed tissue.

Further studies, however, are necessary to validate this approach and in vivo studies are necessary to confirm what has been shown in vitro.

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