STAT Signaling Underlies Difference between Flagellin-induced and Tumor Necrosis Factor-α-induced Epithelial Gene Expression*

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Both bacterial flagellin and the cytokine tumor necrosis factor-α (TNFα) are potent activators of intestinal epithelial cell pro-inflammatory gene expression in general; nonetheless, there seem to be distinct differences in the specific patterns of gene expression induced by these agonists. The goal of this study was to define one such difference and elucidate the signaling mechanism responsible for such differential gene induction by these agonists. We observed that expression of inducible nitric-oxide synthase is substantially induced by flagellin but only minimally expressed in response to TNFα. This difference seemed to be underlain by differential induction of signal transducers and activators of transcription (STAT) activation in that, whereas flagellin and TNFα seemed to be equipotent activators of p38 mitogen-activated protein kinase and nuclear factor-κB, flagellin induced substantially higher levels of STAT-1 and -3 tyrosine phosphorylation. Such flagellin-induced STAT activation exhibited delayed kinetics and was ablated by treatment with cycloheximide. Flagellin-induced activation of STAT-3 was abolished by neutralizing antibodies to interleukin (IL)-6, but not interferon (IFN)β nor IFNγ; none of these neutralizing antibodies had any effect on flagellin-induced STAT-1 tyrosine phosphorylation. Flagellin induced substantially more IL-6 expression than did TNFα, but neither agonist elicited detectable levels of IFN expression. Flagellin-induced expression of inducible nitric-oxide synthase but not IL-6, was abolished by blocking STAT activation with AG490, and was reduced by blocking STAT-3 activation with anti-IL-6. Together, these results indicate that epithelial cell induction of flagellin-specific gene expression is mediated, in part, by STAT activation that results from autocrine activation via IL-6.

The epithelial cells that line the gastrointestinal tract are the front line of defense against the diverse population of commensal and potentially pathogenic microbes that thrive within the lumen of the human intestine. In accordance with this role, in response to pathogens such as the gastroenteritis-causing pathogen Salmonella typhimurium, the intestinal epithelium activates the transcription of a panel of several hundred genes whose overall function is to promote and regulate host defense (1). The necessary and sufficient molecular trigger of this switch in epithelial gene program is the ligation of basolateral toll-like receptor 5 (TLR5) by flagellin monomers, the subunits of bacterial flagella (2–4). Such flagellin-induced changes in epithelial gene expression probably play a key role in promoting the immune inflammatory response that is the histopathologic hallmark of human salmonellosis and serves to clear this infection from the intestinal mucosa while avoiding widespread systemic dissemination in human hosts (5).

The general pattern of gene expression induced by flagellin bears substantial similarity to that induced by the endogenous pro-inflammatory stimulus TNFα; nonetheless, there seem to be significant differences in these patterns, particularly with respect to some genes with important immune function; for example, as shown herein, induction of inducible nitric-oxide synthase (iNOS) was much greater in response to flagellin than to TNFα. The goal of this study was to ascertain the differences in signaling mechanisms that underlie these differences in gene expression. Because we have shown previously that flagellin and TNFα seem to be similar in their abilities to activate 2 central pro-inflammatory signaling pathways, namely nuclear factor-κB and p38 mitogen-activated protein kinase (3, 6), we particularly focused on the signal transducer and activator of transcription (STAT) family of proteins, because STATs are known to play a role in regulation of iNOS, and the differential STAT activation induced by TLR2 and TLR4 ligands underlies the differential gene expression induced by these ligands in macrophages (7). We report that, indeed, the differential STAT activation seems to underlie some of the differences between the specific patterns of gene expression induced by flagellin and TNFα.

EXPERIMENTAL PROCEDURES

Materials—Antibodies were purchased from the following sources; phosphorylated p38, pIgBα, and STATs were from Cell Signaling Technology (Beverly, MA), except for anti-phospho-serine STAT-1, which was from Upstate Biotechnology. iNOS antibody was from R&D Systems, Inc. β-actin antibody was from Sigma. Neutralizing antibody to IFNβ and IFNγ were from Biotrend (Cologne, Germany) and R&D Systems, respectively. Recombinant cytokines (TNFα, IL-6, IFNγ, and IFNβ) and neutralizing antibodies INFγ and the IL-6 receptor were from R&D Systems, Inc. Flagellin was purified from S. typhimurium-conditioned media by anion/cation exchange chromatography and purity verified as described previously (2). In brief, such flagellin does not activate any TLR other than TLR5 (3) and has less than 0.5 pg of LPS/μg of flagellin (8). All other reagents were purchased from Sigma.

1 The abbreviations used are: TLR, toll-like receptor; TNF, tumor necrosis factor; iNOS, inducible nitric-oxide synthase; STAT, signal transducers and activators of transcription; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; Jak, Janus kinase.

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STAT Signaling in Flagellin-induced Gene Expression

35211


cell line. Generation of aflagellate *S. typhimurium* (Fl*ΔC/FΔB*) was described previously (9).

**Cell Culture/Stimulations**—Intestinal epithelial T84 cells were cultured on standard tissue culture plates or, where indicated, collagen-coated permeable supports as described previously (10). These cells consistently grow to very high and uniform confluence, which allows generation of lysates with very uniform protein concentration, which is nonetheless verified via β-actin immunoblots (see below). TNFs and flagellin were used at 20 and 100 ng/ml, respectively, based on our previous extensive studies that have in general defined these concentrations as being about two times saturating, although precise saturating concentrations can vary with cell passage (1–3, 6). Secretion of IL-8 was measured as described previously (11).

**iNOS mRNA Measurement**—iNOS mRNA level was determined by hybridization-based Quantikine mRNA assay kit (R&D Systems). Cells were treated as indicated, total RNA was isolated, and 5 μg per sample was used to quantitate iNOS mRNA following the manufacturer’s protocol.

**SDS-PAGE Immunoblotting**—T84 cells were stimulated as described in figure legends, rinsed in ice-cold Hank’s balanced salt solution, lysed in radioimmunoprecipitation assay buffer (20 mM Tris-HCl, 2.5 mM EDTA, 1% Triton X-100, 10% glycerol, 1% deoxycholate, 0.1% SDS, 50 mM NaF, 10 mM Na3PO4, and 2 mM NaVO4, plus proteinase inhibitors) at a concentration of 106 cells/ml cleared by centrifugation (10 min at 15,000 × g at 4°C), and subjected to SDS-PAGE at 50 μg of protein/lane. Membranes were blocked to specific antibodies. Equal protein loading was verified by stripping each blot and reblotting with anti-β-actin to probe levels of β-actin, a protein whose expression does not change in response to short-term (i.e. hours) pro-inflammatory stimuli (1). Each Western blot shown was representative of more than three similar experiments. Quantification was performed via a Bio-Rad GS 800 Imaging Densitometer following the manufacturer’s instructions. The instrument was set in linear mode and calibrated using the manufacturer’s Calibration TARGET kit combined with recommended Quantity One software. Furthermore, effective linearity was verified by analysis of sequential dilutions of the samples that provided the strongest signal for each set of experiments. One example of such a series of dilutions is shown in figure 1D. When the relative concentration was plotted versus the quantitative densitometric value in this manner, r2 values always exceeded 0.90.

**Cell Fractionation**—After indicated treatment, cells were washed twice with cold phosphate-buffered saline, scraped, centrifuged, and resuspended in cold buffer A (10 mM HEPES, 1.5 mM MgCl2, 10 mM KCl, and 0.5% Nonidet P-40 plus protease inhibitors). Cytosol and nuclei were separated by brief centrifugation (5 min at 500 × g). The nuclear pellet was resuspended and lysed in ice-cold buffer B (20 mM HEPES, 1.5 mM MgCl2, 420 mM NaCl, and 0.2 mM EDTA plus protease inhibitors). Clear nuclear extract was collected after centrifugation (10 min at 15,000 × g).

**Cytokine Neutralization and Jak2 Inhibition**—T84 cells were incubated in serum-free media containing neutralizing antibodies for 1 h before stimulation. The concentrations used were based on manufacturer’s recommendations and our tests of efficacy using recombinant cytokines. In particular, we observed that more than 90% of cytokine activity was blocked at 1:1000 dilutions of these antibodies (see “Results” for discussion of negative controls). After 1 h of incubation, T84 cells were treated with flagellin for 2 h or cytokines for 15 min. Cells were lysed with radioimmunoprecipitation assay buffer and lysates were subjected to immunoblotting. For Jak2 inhibitor AG490 experiment, T84 cells in serum-free medium were pretreated with 10 μM AG490 and then stimulated with 100 ng/ml flagellin for 2 h (for STAT tyrosine phosphorylation detection) or 5 h (for iNOS detection).

**IL-6 Assessment**—IL-6 was measured via by enzyme-linked immunosorbent assay kit using 2 μg of protein in the supernatants of these cultures and maintained for 6–24 h. Only those samples that could be detected at the earliest assayed time point (30 min) and maintained for 2–6 h (Fig. 2A). Quantitation via densitometry indicated that, at 1 h after stimulation, inductions in IL-6 were 8 times as high as those obtained with TNFα and flagellin (Fig. 1G) (2), consistent with many studies showing that IL-8 expression is regulated by these pro-inflammatory signals.

**STAT Phosphorylation Was Differentially Induced by Flagellin and TNFα**—To investigate the signaling mechanisms that might underlie epithelial activation of flagellin-enhanced gene expression, we examined activation of the STAT pathway, because differential activation of STAT-1 underlies TLR4-specific gene expression in macrophages (7). STAT activation was assessed by measuring its phosphorylation status and subcellular localization. First, we examined serine phosphorylation of STATs 1 and 3, because such serine phosphorylation is thought to be important in generating maximal STAT transcriptional activity. TNFα and flagellin induced similar levels of serine phosphorylation of STATs 1 and 3 as assessed by visual examination of films from several experiments. In particular, although neither agonist seemed to induce dramatic changes in serine phosphorylation of either STAT, both agonists consistently induced modest changes in STAT serine phosphorylation that were detectable at the earliest assayed time point (30 min) and maintained for 2–6 h (Fig. 2A). Quantitation via densitometry indicated that, at 1 h after stimulation, inductions in STAT serine averaged, for flagellin and TNFα, respectively, 1.5 ± 0.4-fold and 1.6 ± 0.3-fold for STAT-1 and 1.4 ± 0.3-fold and 1.4 ± 0.2-fold for STAT-3, consistent with our visual as-
Fig. 1. Flagellin induces more iNOS expression in epithelial cells than does TNFα. T84 epithelial cells were stimulated with doubly saturating concentrations of TNFα (20 ng/ml) or flagellin 100 ng/ml (except in C) as described under “Experimental Procedures.” A, at the indicated time, RNA was isolated and iNOS mRNA was quantitated by a hybridization based assay kit as described under “Experimental Procedures.” B, C, and D, iNOS and β-actin were analyzed by immunoblot. E, phospho-p38 immunoblot. F, Phospho-IκBα immunoblot. G, IL-8 secretion, ng/10^6 cells.
assessment that we did not observe consistent differences in the patterns of STAT serine phosphorylation induced by these agonists. Next, we measured STAT tyrosine phosphorylation, because STAT tyrosine phosphorylation is generally accepted to be the rate-limiting event in the promotion of STAT-mediated transcription. In contrast to their similar induction of STAT serine phosphorylation, visual assessment of films of developed SDS-PAGE immunoblots indicated that flagellin consistently induced substantially higher levels of tyrosine phosphorylation of STAT 1 and 3 than did TNFα (Fig. 2A). For both of these stimuli, tyrosine phosphorylation exhibited a relatively delayed time course requiring a minimum of 2 h to observe an

![Diagram of STAT Signaling](image)

**Fig. 2.** Flagellin induces more STAT tyrosine phosphorylation than TNFα. Epithelial cells were stimulated with flagellin or TNFα for the indicated times. A, cells were lysed and assayed for STAT phosphorylation by SDS-PAGE immunoblotting. FY, phosphotyrosine; PS, phosphoserine. B and C, cells were disrupted, fractionated as described under “Experimental Procedures,” and total STAT (B) and phosphotyrosine STAT (C) were measured in fractions.

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Procedures.” B and C, cells were lysed at the indicated time (B) or 5 h (C), and iNOS protein was measured via SDS-PAGE/immunoblotting. D, lysates from C, control sample, and those showing the highest induction for each stimuli (60 ng/ml flagellin or 80 ng/ml TNFα) were diluted as indicated with lysis buffer and re-blotted to aid in quantitation of differential induction and demonstrate that the signal was in the working linear range of the film. E and F, cells were lysed at the indicated times and levels of phospho-p38 and phospho-IκBα were assayed by SDS-PAGE/immunoblotting. G, cell supernatants were isolated at indicated time after stimulation and IL-8 secretion quantitated by enzyme-linked immunosorbent assay. Results in A and G are the means ± S.E. of three parallel experiments whereas those in B–F are representative of many experiments.
increase relative to untreated epithelial cells. Consistent with our visual assessment, quantitation via densitometry indicated that, at 2 h, inductions in STAT tyrosine phosphorylation averaged, for flagellin and TNFα, respectively, 5.7 ± 1.2-fold and 2.5 ± 0.4-fold for STAT1 and 10.9 ± 4.5-fold and 2.5 ± 1.6-fold for STAT-3. Total levels of these STATs did not change throughout the time course assayed. We also examined the tyrosine phosphorylation of STATs 5 and 6 in response to flagellin and TNFα but did not observe any changes in response to either agonist (data not shown).

Tyrosine phosphorylation regulates STAT dimerization and, subsequently, nuclear translocation (13). Thus, we next assessed STAT nuclear translocation by measuring levels of STATs that were in the cytosolic or nuclear fraction of disrupted epithelial cells (Fig. 2B). In parallel with its greater potency at inducing STAT tyrosine phosphorylation, flagellin induced substantially more nuclear translocation of STAT-3 than did TNFα (average induction by densitometry was 3.2-fold greater for flagellin than TNFα); a similar pattern of activation of STAT-1, albeit less robust, was observed (data not shown). Thus, as shown in Fig. 2C, flagellin treatment resulted in more nuclear tyrosine-phosphorylated STAT-1 and -3 than did treatment by TNFα. For either stimulus (or STAT), we consistently observed a 0.5–1-h delay between STAT tyrosine phosphorylation and nuclear translocation. Thus, flagellin is more potent than TNFα at activating STATs 1 and 3, consistent with the possibility that differential STAT activation may underlie flagellin-enhanced gene expression.
Flagellin-induced STAT-3 Activation Is Mediated by IL-6—

The 2-h delay in appearance of STAT tyrosine phosphorylation in response to flagellin (and TNFα) suggested that newly synthesized proteins mediate this signaling event. In support of this notion, we observed that the protein synthesis inhibitor cycloheximide abolished STAT tyrosine phosphorylation in response to flagellin (Fig. 3A). To rule out the possibility that cycloheximide was blocking STAT phosphorylation by nonspecific means, we verified that cycloheximide had no effect on total STAT levels or on STAT tyrosine phosphorylation induced by either IL-6 or IFNβ (Fig. 3A and data not shown). Thus, flagellin-induced STAT tyrosine phosphorylation requires new protein synthesis.

We next considered some of the candidate molecules that might mediate STAT activation in response to flagellin. Because IL-6 was known to be secreted by epithelia (14) and given its potent ability to activate STATs in epithelia, IL-6 seemed a likely candidate to be the paracrine or autocrine mediator responsible for flagellin-induced STAT activation. Interferons, especially IFNβ, also seemed like reasonable candidate mediators in light of their ability to activate STATs in general and the role of IFNβ in LPS-induced macrophage activation in particular (7). This hypothesis was investigated via the use of cytokine-neutralizing antibodies, the efficacy and specificity of which we first verified (Fig. 3B), and by measuring expression of these cytokines in response to flagellin. In particular, we used both positive and negative controls to test a series of commercially available cytokine neutralizing antibodies to investigate which cytokines might be paracrine mediators of flagellin-induced STAT activation.

We observed that, in our hands, several such purchased antibodies either did not effect neutralize their targets or had nonspecific effects on responses induced by other recombinant cytokines. However, as shown in Fig. 3B, three commercially available antibodies were able to effectively neutralize their targets in that antibodies to IFNβ, IFNγ, and IL-6 (receptor) all blocked STAT activation in response to their corresponding cytokines. It is noteworthy that none of these antibodies blocked responses to other cytokines (anti-IFNβ, anti-IFNγ, and anti-IL-6 did not block STAT activation induced by IFNγ or IL-6, IFNβ or IL-6, or IFNβ or IFNγ, respectively (Fig. 3B and data not shown), indicating that these purified antibodies did not have nonspecific effects on STAT activation. Next, we measured whether such neutralizations modulated STAT activation in response to flagellin as illustrated in Fig. 3C, pretreatment with the IL-6 receptor-neutralizing antibody abolished flagellin-induced STAT-3 tyrosine phosphorylation but had no effect on STAT-1 tyrosine phosphorylation, indicating that IL-6 is required for activation of STAT-3 but not STAT-1 in these cells. Although both IFNβ and IFNγ potently activated STAT-1 tyrosine phosphorylation in epithelial cells and their neutralizing antibodies suppressed that activation, neither neutralizing antibody was able to reduce STAT-1 or -3 tyrosine phosphorylation in response to flagellin, suggesting their lack of involvement in this response.

In light of the apparent requirement of IL-6 for flagellin-induced STAT-3 activation, we next measured secretion of IL-6 in response to flagellin and TNFα (Fig. 4). We observed that flagellin induced substantially more (−3-fold) IL-6 secretion than TNFα, consistent with the notion that differential induction of IL-6 may play a role in induction of flagellin-enhanced gene expression. Although the per-cell level of secretion of IL-6 is relatively low (e.g., about 75-fold less than IL-8), IL-6 secretion (unlike IL-8) is polarized to the apical surface. In our polarized model system, apical volumes are kept relatively small to mimic the very small volume of intestinal crypts. Hence, this leads to concentrations of IL-6 greater than 100 pg/ml and to the likely existence of microgradients closer to the cell surface, thus approaching the 500 pg/ml concentrations of recombinant IL-6 that we observed are sufficient to activate STAT-3 (Fig. 4C). Moreover, specific neutralization of IL-6 blocked STAT-3 activation, demonstrating that this level of IL-6 production is important in the context of flagellin stimulation. In contrast, sensitive enzyme-linked immunosorbent assay kits able to detect picogram quantities of cytokines were unable to detect either IFNγ or IFNβ in response to flagellin (data not shown). Together, these results indicate that IL-6 mediates flagellin-induced STAT-3 activation and that prefer-
ential induction of IL-6 in response to flagellin, compared with TNFα, partly underlies flagellin-enhanced STAT-3 activation and, subsequently, perhaps, flagellin-enhanced gene expression. In contrast to its role in LPS-stimulated macrophages, neither IFNβ nor IFNγ plays a role in flagellin-induced STAT activation of T84 epithelial cells.

In light of our observation that flagellin is a more potent activator of STATs than TNFα, we next investigated the roles of STAT activation in the induction of iNOS, a gene whose expression is enhanced in response to flagellin compared with TNFα. First, we blocked flagellin-induced STAT activation with the use of an IL-6 receptor-neutralizing antibody as described above. This neutralizing antibody partially blocked flagellin-induced iNOS induction (Fig. 5A). To verify that this antibody did not non-specifically block flagellin-induced gene expression per se, we measured its effects on flagellin-induced IL-8 expression and found no effect (data not shown). In contrast to the IL-6 receptor-neutralizing antibody, neutralizing antibodies to IFNβ and IFNγ did not, by themselves, affect flagellin-induced iNOS expression or significantly enhance the attenuation by the IL-6 receptor antibody. Thus, flagellin-induced IL-6-mediated STAT-3 activation seems to modulate iNOS expression in these cells. Next, we examined the role of STAT activation in general using the best available pharmacologic inhibitor of this pathway, the Jak2 inhibitor AG490 (14). AG490 ablated tyrosine phosphorylation of STAT-1 and -3 and abolished iNOS expression in response to flagellin (Fig. 5B). AG490 did not block flagellin-induced gene expression per se in that flagellin-induced IL-6 expression was unaffected by this inhibitor (Fig. 5C). Together, these results suggest that activation of STATs 1 and 3 play a role in regulating flagellin-enhanced gene expression in general and flagellin-induced iNOS expression in particular.

Finally, we sought to define the portion of the overall STAT activation that occurs in response to epithelial colonization by live *S. typhimurium* that could be accounted for by flagellin. First, we examined the pattern of STAT activation elicited in response to apical colonization of model epithelia by this pathogen (Fig. 6). The general pattern was similar, with activation of both STAT-1 and -3, but not other STATS; interestingly, however, the kinetics of the response were slower, possibly because of the time required for flagellin to reach the basolateral membrane, where its receptor TLR5 is located. In contrast, model epithelia colonized by *S. typhimurium* carrying deletions in its two flagellin genes exhibited no STAT activation at any time assayed after colonization. Thus, flagellin is both sufficient and necessary for *S. typhimurium* activation of STAT signaling in intestinal epithelia, paralleling its importance in activating epithelial pro-inflammatory gene expression in general (1, 2).
**DISCUSSION**

As the front line of host defense, intestinal epithelial cells are capable of rapidly changing their patterns of gene expression to allow them to respond to pathogenic challenges encountered in the gastrointestinal tract (15). The trigger for epithelial cells to alter their pattern of gene expression can be the detection of a bacterial product, such as flagellin ligating TLR5, or can result from the secretion of an endogenous mediator, such as TNFα, by a cell that has itself detected an inappropriate presence of a microbe or other danger signal. The general epithelial response to either of these agonists is to express genes whose overall role seems to be to promote an immune inflammatory response (1, 16). In accordance with the possibility that these agonists have somewhat similar effects on epithelial function, there is substantial similarity in the patterns of gene expression induced by these molecules. As such, similar major pro-inflammatory signals, such as the nuclear factor-κB and mitogen-activated protein kinase pathways, are activated by these agonists. However, there are also important differences in some of the specific genes that are or are not activated by these agonists that presumably play an important role in coordinating a functional immune response.

This study focused on defining and understanding one such difference (i.e. that flagellin is a much more potent activator of iNOS). We observed that, mechanistically, this difference results in part from the more potent induction of IL-6 secretion by flagellin compared with TNFα and, as such, IL-6 then acts in concert with a yet-to-be identified factor(s) to activate STAT-3 and -1, thus leading to iNOS expression. This mechanism is paradigmatically similar to the means by which the TLR4 ligand LPS, but not TLR2 ligands, activate iNOS expression in macrophages (7), although the specific mediator(s) involved are different in that whereas IFNβ is required in macrophages, use of the same immunoreagents was able to rule out a role for IFNβ in our epithelial cell system (note that neither LPS nor TLR2 ligands activate iNOS or classic pro-inflammatory genes such as IL-8 in intestinal epithelial cells (17)). This difference between LPS-induced iNOS in macrophages and flagellin-induced iNOS in epithelial cells could be the result of TLR5 and TLR4 using different sets of adaptor proteins or could reflect an inherent difference in that the IFN genes are seemingly quiescent (regardless of stimulus) in epithelial cells. The differential activation of STAT-1 and -3 between flagellin and TNFα was exhibited only on the tyrosine phosphorylation site that is responsible for STAT protein dimerization and nuclear translocation. In contrast, no significant difference on STAT serine phosphorylation was observed between flagellin and TNFα stimulation. This suggests that levels of nuclear translocation of these transcription factors, rather than maximal activity perse, is responsible for the differential induction of iNOS and possibly other flagellin-enhanced genes.

In contrast to the role of IL-6 in mediating STAT-3 activation, the mediator responsible for STAT-1 activation remains unknown. It is likely to be quite important for induction of flagellin-enhanced genes, such as iNOS, as evidenced by our observation that blocking both STAT-1 and -3 with AG490 completely blocked iNOS induction, whereas blockade of STAT-3 via anti IL-6 only partially attenuated induction of this gene. Besides excluding a role for IFNβ and IFNγ as flagellin-induced mediators of STAT-1, we have also ruled out roles for IL-8, IL-1β, and IFNα. Thus, the precise mediator(s) remain under investigation in our laboratory.

Why flagellin is a more potent inducer of IL-6 secretion than TNFα is unclear, because the nuclear factor-κB and p38 signaling pathways are thought to be the primary regulators of IL-6 expression (18), and these seem to be activated similarly in response to these agonists. It seems likely that besides these canonical pro-inflammatory signaling pathways, flagellin also regulates IL-6 expression via some of the additional elements known to be present in the IL-6 promoter. One possibility might be activation of the IL-6’s promoter activating transcription factor/CAMP response element-binding protein sites, which would become used upon activation of the cyclic AMP (19) pathway that has been shown to occur in epithelial cells in response to some agonists (e.g. adenosine) (12). Another possibility might be a Ca2+–induced IL-6 expression, in that Ca2+-mobilizing agonists are potent and rapid activators of IL-6 in hematopoietic cells (20), and elevations in intracellular [Ca2+] occur in response to flagellin (21) and live S. typhimurium but not TNFα (12). Determination of which, if any, of these mechanisms may underlie our current observations remains an important challenge in this area.

The functional significance of why the epithelium would activate iNOS expression in response to flagellin but not TNFα is not entirely clear, but a reasonable possibility is that epithelial activation of iNOS in response to an infectious agent may play a role in altering vascular tone to reduce blood flow in the infected area, thus slowing the spread of bacteria to systemic organs. In humans, salmonellosis is generally restricted to the intestinal mucosa despite the ability of S. typhimurium to readily invade epithelial cells and survive in macrophages, and reduced blood flow out of the infected area seems likely to play a role in the containment process. Such alteration in blood flow may not be as generally necessary in all situations in which TNFα is a mediator; thus, perhaps TNFα does not potently up-regulate iNOS expression in gut epithelial cells for this...
reason. It is likely that as microarray technology continues to advance, more and more examples of differential gene activation by various pro-inflammatory agonists will emerge. Such differential gene expression and its underlying signaling mechanisms are likely to be essential to the long term outcome of many infectious and immunological processes, and further understanding of them may permit therapeutic manipulations to promote human health.

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