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Stereotypic and specific elements of the human colonic response to *Entamoeba histolytica* and *Shigella flexneri*

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Summary

The clinical presentations of bacillary dysentery caused by shigella, and amoebic dysentery caused by the protozoan parasite *Entamoeba histolytica,* can be indistinguishable, with both organisms causing colonic mucosal damage and ulceration. However, the two organisms are quite distinct, and have very different pathogenic mechanisms. This raises the fundamental question of whether the similar clinical manifestations reflect a stereotypic response of the human gut to mucosal injury, or whether there are differences at the molecular level in the host response to individual gut pathogens. To characterize the human colonic response to each pathogen at the molecular level, we measured the differential transcription of nearly 40 000 human genes in sections of human colonic xenografts obtained 4 and 24 h following infection with *Shigella flexneri* or *E. histolytica*. Our results indicate that much of the human colonic response to these two pathogens is stereotypic, with increased expression of genes activated in cells undergoing stress and/or hypoxic responses, genes encoding cytokines, chemokines, and mediators that are involved in immune and inflammatory responses, and genes encoding proteins involved in responses to tissue injury and in tissue repair. The responses to amoeba and *Shigella* were not identical however, and we found unique elements in each response that may provide new insights into the distinct pathogenic mechanisms of *E. histolytica* and *S. flexneri*.

Introduction

Bacillary dysentery, caused by *Shigella* spp., and amoebic dysentery, caused by the protozoan parasite *Entamoeba histolytica*, are major threats to public health worldwide. Both *E. histolytica* and *S. flexneri* specifically infect human colon, resulting in colitis, with mucosal ulceration and inflammation. Whereas the clinical manifestations of bacillary dysentery and amoebic dysentery are often indistinguishable, the two organisms have very different pathogenic mechanisms. *Shigella* are primarily intracellular pathogens that invade into intestinal epithelial cells, multiply intracellularly, kill the host cell, then spread horizontally into adjacent epithelial cells, or into the lamina propria. (Tran *et al*., 2000). In contrast, *E. histolytica* is an extracellular pathogen that ingests, but does not reside within, host cells. Instead, *E. histolytica* trophozoites lyse human cells by the action of pore-forming molecules, and digest and invade through the host extracellular matrix by secreting cysteine proteinases. (Stanley, 2003) Despite these differences, there are common elements in colitis caused by *Shigella* and colitis secondary to *E. histolytica* infection, as in both infections, the host inflammatory response appears to be a critical component of disease. Mucosal biopsy samples from individuals with *Shigella* show increased levels of proinflammatory mediators such as IL-8, IL-1β, and TNF alpha. (Raqib *et al*., 1995) In studies of *Shigella flexneri* and *E. histolytica* infections in human colonic xenografts in severe combined immunodeficient mice (SCID-HU-INT mice), both *E. histolytica* and *Shigella* induced intestinal epithelial cell production of cytokines and chemokines, including IL-8 and IL-1β (Seydel *et al*., 1998; Zhang *et al*., 2001). In both infections, the transcription factor NF-κB may play a key role in mediating the inflammatory response. In the SCID-HU-INT model of *E. histolytica* infection, NF-κB was required for activation of cytokine production in intestinal epithelial cells, the resultant neutrophil influx into the gut mucosa, and the overall inflammatory response that led to tissue damage. (Seydel *et al*., 1998) *Shigella* invasion into human epithelial cells rapidly activates NF-κB and cytokine production within those cells, through unique LPS-dependent pathways (Philpott *et al*., 2000).

The finding that two very different pathogens, *E. histolytica* and *S. flexneri*, as well as other invasive bacteria, may induce gut inflammation through similar pathways, raises fundamental questions about the nature of the gut response to enteric pathogens. Do all pathogens that damage intestinal epithelial cells induce the same stereotypic response within the colon? Or are there pathogen-specific responses – pathways for inflammation, host defence, or injury repair that may be induced only by

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particular types of organisms? To address this question, we performed a transcriptional analysis, using infection of SCID-HU-INT mice to compare the human colonic response to *E. histolytica* and *S. flexneri* infection. We measured the differential transcription of nearly 40 000 human genes in sections of human colonic xenografts at 4 and 24 h following infection with *S. flexneri* or *E. histolytica*. Our results indicate that much of the human colonic response to these two pathogens is similar, and that the pattern of differentially expressed genes resembles those seen with inflammatory bowel disease, suggesting that the gut has a limited repertoire of responses to mucosal injury. The responses to amoeba and *Shigella* were not identical however, and we find unique elements in each response that may provide new insights into the distinct pathogenic mechanisms of *E. histolytica* and *S. flexneri*.

Results

Histologic appearance of E. histolytica *or* S. flexneri *colonic infection at 4 and 24 h*

Examples of histologic findings from human colonic xenografts with amoebic or shigella infection at 4 and 24 h are shown in Fig. 1. *Entamoeba histolytica* infection at 4 h

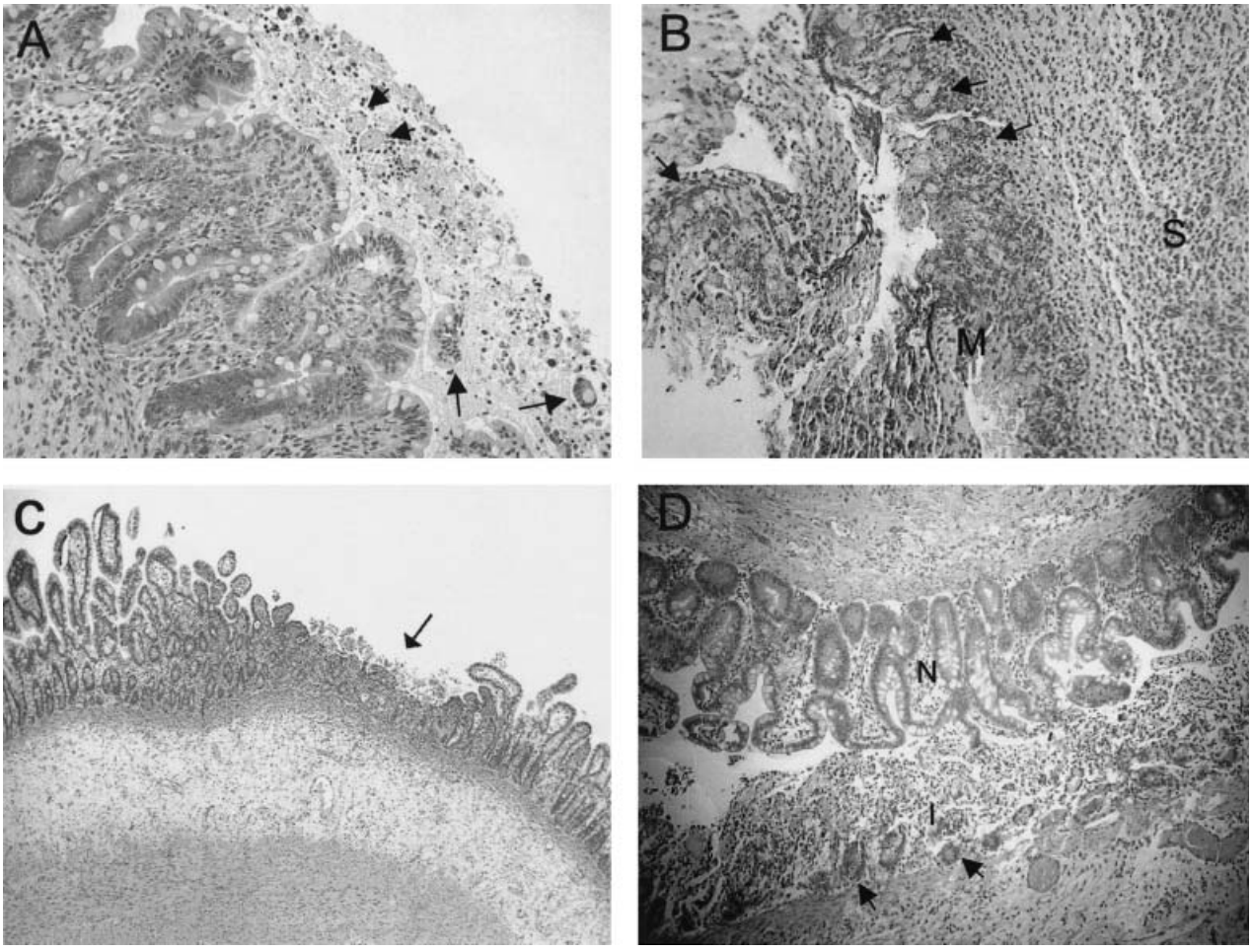

Fig. 1. Photomicrographs of *E. histolytica* or *S. flexneri* infected human colonic xenografts at 4 or 24 h after infection.
A. Section of a human colonic xenograft infected for 4 h with *E. histolytica* trophozoites. Amoebic trophozoites (arrowheads) are seen in the lumen with surrounding cellular debris and a minimal inflammatory response. Some mucosal damage with denuded portions of villi are seen (large arrows). Magnification 250×.
B. Section of a human colonic xenograft infected for 24 h with *E. histolytica* trophozoites showing ulceration, marked inflammation and tissue destruction. There is almost complete loss of normal architecture in the mucosa (M), and masses of *E. histolytica* trophozoites are visible (arrowheads) throughout the mucosa and in regions of detached mucosa in the lumen. The submucosa (S) has been infiltrated by polymorphonuclear cells. Magnification 200×.
C. Section of human colonic xenografts infected for 4 h with *S. flexneri*. An ulcer with mucosal disruption and hypercellularity is visible underneath the arrow. Mucosal haemorrhage is present, and erythrocytes and neutrophils are present in the lumen. Magnification 200×.
D. Section of a human colonic xenograft infected for 24 h with *S. flexneri*. Two opposing layers of colonic mucosa are shown, one relatively spared (N) and the other markedly involved (I), with loss of normal architecture, 'ghost' villi, intense polymorphonuclear cell infiltration extending into the lumen, and mucosal haemorrhage. Arrows indicate villous and crypt remnants. The submucosal region is relatively spared. Magnification 200×.

is associated with some mucosal damage, the presence of a few inflammatory cells in the colonic lumen, and minimal to no invasion into submucosal tissues (Fig. 1A). At 24 h of infection, *E. histolytica* has caused significant mucosal damage, with amoebic trophozoites invading into submucosal tissues, some mucosal hemorrhage, and a marked inflammatory infiltrate, primarily neutrophils, in the submucosal region (Fig. 1B). *Shigella flexneri* infection at 4 h causes some mucosal damage associated with mucosal inflammation and some mucosal haemorrhage, but submucosal tissues appear uninvolved (Fig. 1C). At 24 h of infection, *S. flexneri* infection has caused diffuse mucosal damage, with only a few remnants of normal mucosa remaining in involved areas, marked hemorrhage, but relatively little evidence for submucosal invasion (Fig. 1D). These data indicate that the inflammatory response and the levels of mucosal damage should be most marked in *E. histolytica* infection at 24 h and *S. flexneri* infection at 4 and 24 h.

Comparative transcriptional analysis of E. histolytica *and* S. flexneri *colonic infection at 4 and 24 h: genes showing increased expression in both* E. histolytica *and* S. flexneri *infection*

The complete data for all genes in all the experiments performed using the Stanford arrays and the confirmatory Incyte experiments are available at http://stanleylab.wustl.edu/ehmd. We found 148 genes that showed increased expression in both *E. histolytica* and *S. flexneri* infection compared to uninfected human colonic xenografts at one or both of the two time-points (data for each of the experiments is shown in Fig. 2, whereas mean values and gene groupings are shown in Table 1). Of these 148 genes, 81 were represented on the Incyte Unigene-based array, and 61/81 (75%) showed the same expression pattern in the confirmatory experiment (marked 'C' for confirmed next to the gene name) (Fig. 2). The 148 genes expressed at higher levels in both *S. flexneri* and *E. histolytica* infection fell into several categories (Table 1). Many were genes known to be activated in cells undergoing stress and/or hypoxic responses [e.g. nine members of the metallothionein family (Davis and Cousins, 2000), c-Jun and c-Fos family members (Weston and Davis, 2002), hypoxia-inducible factor-1 (HIF-1) (Huang and Bunn, 2003), immediate early response 3, serum amyloid proteins (He and Sang, 2003), DAF (Andoh *et al*., 2001), heat-shock proteins, growth-arrest and DNA-damage-inducible 3 (Bosio *et al*., 2002), alpha crystallin (Narberhaus, 2002), vacuole-membrane protein (Dusetti *et al*., 2002)]. Previous studies in the SCID-HU-INT model had revealed significant increases in the expression of genes encoding IL-1β, IL-8, and COX-2 with *E. histolytica* infection, and increases in IL-1β and IL-8 in *S. flexneri* infection (Seydel *et al*., 1997; Stenson *et al*., 2001; Zhang *et al*., 2001). These findings were confirmed in the microarray analysis, and the expression of genes encoding a number of other cytokines, chemokines and mediators that are involved in immune and inflammatory responses was also found to be increased in both *E. histolytica* and *S. flexneri* infection (Table 1). This included the interleukin-1 receptor, interleukin-6, multiple TNF-α-induced proteins, several interferon γ-induced proteins, all 3 GRO-family members, superoxide dismutase 2, monocyte chemotactic protein (MCP)-1, MCP-2, MCP-3, ENA-78, MIP-3α, leukaemia-inhibitory-factor, lipocalin 2 (Devireddy *et al*., 2001), and leukotriene C4 synthase. Many of the genes expressed at higher levels in *E. histolytica* and *S. flexneri* infection encode proteins involved in responses to tissue injury and in tissue repair and remodelling (e.g. matrix metalloproteinase (MMP) 1, MMP12, MMP17 (Vincenti and Brinckerhoff, 2001), tissue inhibitor of metalloproteinase 1 (TIMP1), tissue factor pathway inhibitor 2, cryptic protein, connective tissue growth factor, cysteine-rich angiogenic inducer, angiopoeitin-like 4, stanniocalcin 1, thrombospondin 1, ICAM-1, tenascin, TGF-β induced protein 68 (kDa). The increased expression of several genes encoding metabolic enzymes known to be expressed in response to hypoxia (e.g. lactate dehydrogenase A, enolase 1 and 2, fructose bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase) (Semenza *et al*., 1996; Lu *et al*., 2002) or glucocorticoids (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3) (Lange *et al*., 1992) was also found.

As shown in Fig. 2, most of the 148 genes expressed at higher levels in human colonic xenografts infected with amoeba or shigella showed increased expression at all four time-points, or at the three time-points where colonic inflammation and tissue destruction was most prominent – amoebic infection at 24 h and shigella infection at both 4 and 24 h. There were a few genes that appeared to be specifically expressed late in *E. histolytica* and *S. flexneri* infection (e.g. interleukin-11, serine/cysteine proteinase inhibitor clade E, COX-2), and a few genes that were expressed only early in both *E. histolytica* and *S. flexneri* infection (e.g. junD, protein phosphatase 2 catalytic subunit, protein tyrosine phosphatase type IVA member 1, interferon-stimulated protein 15 kDa). In general, among individual genes showing increased expression in both *E. histolytica* and *S. flexneri* infection, differential expression ratios were higher in the *S. flexneri*-infected human colonic xenografts (Fig. 2).

Genes showing increased expression in S. flexneri *infection but not* E. histolytica *infection*

We found 81 genes that met criteria for increased

Fig. 2. Genes differentially expressed in both *E. histolytica* and *S. flexneri* infected human colonic xenografts at 4 and/or 24 h of infection. Gene expression profiles from three experiments looking at *E. histolytica* infection at 4 h (A4), three experiments looking at *E. histolytica* infection at 24 h (A24) and three experiments looking at *S. flexneri* infection at 4 h (S4) or 24 h (S24) are shown. Genes are listed by gene name, and those genes showing a similar expression pattern in the confirmatory experiments using the Unigene array are marked by a (C). Those genes that were represented on the Unigene array but did not exhibit the same expression pattern are marked as (NC). The data are expressed as the $\log_2$ of the expression ratio, and the key for intensity of expression is: 

Grey bars signify missing values secondary to failure of a given gene spot to meet minimal intensity requirements for that experiment.

Table 1. Genes showing increased expression in both *E. histolytica* and *S. flexneri* infection at one or both time-points.

Gene name	Accession	A4	A24	S4	S24
Stress response					
apolipoprotein E	T70891	1.8 ± 0.19	1.71 ± 0.29	2.88 ± 0.32	2.09 ± 0.12
cold shock domain protein A	AA455300	0.99 ± 0.21	2.27 ± 0.21	3.97 ± 2.19	5.53 ± 1.35
crystallin, alpha B	AA504891	2.32 ± 0.16	2.45 ± 0.01	4.43 ± 0.87	9.54 ± 3.71
DNA2 DNA replication helicase 2-like (yeast)	AI248069	3.24 ± 0.6	2.27 ± 0.44	9.84 ± 4.83	7.47 ± 2.24
EST, Moderately similar to Cd-7 metallothionein-2 [H.sapiens]	R16539	7.99 ± 2.25	3.08 ± 0.46	5.31 ± 2.34	7.65 ± 1.56
growth arrest and DNA-damage-inducible, beta	AA504354	6.83 ± 2.14	2.36 ± 0.37	3.64 ± 0.61	8.46 ± 4.44
H.sapiens mRNA for metallothionein isoform 1R	AI289110	7.34 ± 1.3	3.44 ± 0.52	19.61 ± 1.14	18.65 ± 3.55
haptoglobin	AI985788	2.26 ± 0.45	18.8 ± 12.4	3.01 ± 1.03	26.12 ± 19.01
heat shock 70 kDa protein 1-like	H17513	1.42 ± 0.24	1.74 ± 0.14	1.3 ± 0.59	21.22 ± 11.18
HIF-1 responsive RTP801	AA447746	5.09 ± 1.48	3.82 ± 0.91	3.43 ± 1.48	10.04 ± 4.12
Homo sapiens clone MT1Y metallothionein 1Y mRNA, complete cds	R06601	12.77 ± 3.4	4.28 ± 1.5	18.12 ± 8.91	22.4 ± 3.66
hypoxia-inducible factor 1, alpha subunit	W47003	3.43 ± 0.44	1.73 ± 0.28	3.24 ± 0.61	7.26 ± 1.84
immediate early response 3	AA457705	2.48 ± 0.75	7.63 ± 3.57	5.66 ± 2.98	28.17 ± 16.81
likely orthologue of rat vacuole membrane protein 1	AA159669	3.24 ± 1.22	1.52 ± 0.3	1.69 ± 0.22	3.82 ± 0.35
metallothionein 1F	N55459	8.28 ± 2.97	3.9 ± 1.16	25.82 ± 3.24	39.83 ± 19.47
metallothionein 1G	H53339	7.33 ± 1.59	2.65 ± 0.24	49.67 ± 25.82	13.87 ± 6.69
metallothionein 1H	H77597	7.47 ± 3.19	11.5 ± 2.59	34.59 ± 17.84	11.75 ± 0.43
metallothionein 1 l	N80129	8.92 ± 0.95	5.04 ± 1.17	25.7 ± 11.24	17.54 ± 5.05
metallothionein 2 A	AA156031	10.76 ± 1.57	4.32 ± 0.56	40.38 ± 2.66	42.36 ± 0.75
metallothionein 3 [growth inhibitory factor (neurotrophic)]	AI362950	6.38 ± 2.46	6.08 ± 0.96	13.99 ± 4.6	15.66 ± 2.56
serum amyloid A1	H25546	2.83 ± 0.72	8.36 ± 2.97	10.78 ± 5.23	47.18 ± 4.49
serum amyloid A2	AI659145	17.13 ± 11.43	28.99 ± 9.18	6.02 ± 3.08	41.05 ± 8.25
serum amyloid A4, constitutive	AI344545	5.64 ± 0.12	21.67 ± 6.73	– ± –	30.37 ± 22.25
uncoupling protein 2 (mitochondrial, proton carrier)	H61242	4.52 ± 1.2	1.45 ± 0.21	6.06 ± 1.51	9.28 ± 5.05
Immune response/Inflammatory mediators/Cytokines/Chemokines					
ENA-78	AA878880	5.23 ± 1.41	3.97 ± 0.72	2.13 ± 0.47	7.42 ± 2.9
decay accelerating factor for complement (CD55)	R09561	4.15 ± 0.60	3.22 ± 0.44	2.48 ± 0.27	2.01 ± 1.04
GRO1 oncogene (melanoma growth stimulating activity, alpha)	W42723	8.63 ± 2.41	12.65 ± 3.98	9.57 ± 2.41	38.17 ± 2.71
GRO2 oncogene	R47771	7.32 ± 3.16	3.88 ± 0.81	4.92 ± 2.29	23.22 ± 10.03
GRO3 oncogene	AA935273	6.4 ± 2.84	6.02 ± 1.63	7.69 ± 3.58	25.18 ± 6.74
homologous to PBEF	AA281932	5.82 ± 0.45	3.82 ± 0.66	8.86 ± 2.68	15.73 ± 2.2
hypothetical SBBI03 protein (homology with TRAF-2)	AI299601	7.81 ± 4.81	4.09 ± 0.77	21.71 ± 6.57	14.4 ± 0.78
interferon induced transmembrane protein 3 (1–8 U)	AA464416	1.57 ± 0.22	2.22 ± 0.16	2.95 ± 0.67	4.16 ± 0.69
interferon regulatory factor 7	AA477347	2.89 ± 0.03	1.73 ± 0.27	6.25 ± 0.6	6.56 ± 2.7
interferon stimulated gene (20 kDa)	AA150500	4.63 ± 0.39	2.62 ± 0.16	1.43 ± 0.66	3.92 ± 0.61
interferon-stimulated protein, 15 kDa	AA120862	4.32 ± 2.33	0.68 ± 0.1	4.24 ± 1.63	0.6 ± 0.26
interleukin 1 receptor type 1	R56687	2.03 ± 0.78	1.80 ± 0.24	3.97 ± 0.85	8.17 ± 4.45
interleukin 1, beta	W47101	1.35 ± 0.54	12.43 ± 1.97	4.14 ± 0.58	13.07 ± 8.29
interleukin 11	AI148233	0.94 ± 0.41	19.84 ± 4.02	0.52 ± 0.1	15.22 ± 4.07
interleukin 4 receptor	AA292025	2.91 ± 1.07	1.01 ± 0.34	1.27 ± 0.42	2.41 ± 0.87
interleukin 6 (interferon, beta 2)	N98591	1.51 ± 0.59	8.92 ± 6.28	2.97 ± 0.32	17.79 ± 2.38
interleukin 8	AA082747	1.66 ± 0.44	1.64 ± 0.31	11.34 ± 10.66	5.83 ± 0.56
leukaemia inhibitory factor (cholinergic differentiation factor)	R50018	1.26 ± 0.66	4.08 ± 1.29	4.63 ± 3.17	7.55 ± 1.36
leukotriene C4 synthase	AI299075	3.62 ± 0.89	3.43 ± 0.98	4.9 ± 2.51	5.07 ± 0.7
lipocalin 2 (oncogene 24p3)	AA400973	2.43 ± 0.54	7.61 ± 2.36	2.95 ± 0.85	6.9 ± 2.89
MIP-3a (small inducible cytokine subfamily A (Cys-Cys), member (20)	AI285199	18.78 ± 1.88	5.32 ± 2.2	3.67 ± 0.48	5.85 ± 3.36
monocyte chemotactic protein 1	AA425102	3.96 ± 0.49	2.5 ± 0.29	25.56 ± 7.19	40.98 ± 20.33
monocyte chemotactic protein 2	AI268937	1.44 ± 0.73	2 ± 0.66	12.15 ± 4.36	10.6 ± 2.58
monocyte chemotactic protein 3	AA040170	2.53 ± 0.19	2.05 ± 0.81	5.17 ± 2.48	3.66 ± 1.5
pre-B-cell colony-enhancing factor	AI335002	2.72 ± 0.17	1.38 ± 0.45	5.26 ± 0.45	6.28 ± 0.42
COX-2	R80217	0.88 ± 0.43	8.47 ± 1.72	5.19 ± 2.56	9.81 ± 0.49
prostate differentiation factor (MIC-1)	AA450062	5.35 ± 1.82	3.1 ± 0.96	2.25 ± 0.53	10.3 ± 2.42
S100 calcium binding protein A9 (calgranulin B)	AA864554	0.7 ± 0.12	2.32 ± 0.31	0.9 ± 0.39	9.7 ± 3.87
superoxide dismutase 2, mitochondrial	W78148	5.15 ± 0.82	3.53 ± 0.48	7.06 ± 1.92	13.43 ± 2.63
tumor necrosis factor, alpha-induced protein 3	AA433807	7.84 ± 1.53	3.59 ± 0.1	14.64 ± 7.16	18.83 ± 10.44
type I transmembrane protein Fn14 (TWEAK receptor)	AI221536	7.31 ± 3.44	3.7 ± 0.21	4.42 ± 1.52	3.88 ± 0.7
vasoactive intestinal peptide	AI217172	0.6 ± 0.1	2.4 ± 0.35	5.63 ± 4.17	8.55 ± 2.14
Growth factors/Tissue Remodeling/Cell Cycle Regulators/Apoptosis-related/Oncogenes					
adipose differentiation-related protein	AA142916	2.9 ± 0.73	1.61 ± 0.26	3 ± 0.51	8.16 ± 1.18
angiopoietin-like 4	T53705	2.37 ± 0.12	12.09 ± 4.61	1.13 ± 0.66	6.94 ± 1.63
baculoviral IAP repeat-containing 5 (survivin)	AA460685	5.42 ± 2.01	3.06 ± 0.43	18.26 ± 4	15.39 ± 5.57
BCL2-associated athanogene 3	AI371684	3.33 ± 1.14	1.52 ± 0.42	6.74 ± 0.91	4.9 ± 2.53
colony stimulating factor 1 receptor	R92609	2.71 ± 0.84	1.03 ± 0.26	3.87 ± 1.13	4.08 ± 1.06
connective tissue growth factor	AA598794	5.88 ± 0.09	3.06 ± 0.18	15.26 ± 9.25	7.3 ± 2.49
cryptic gene	AA878309	5.34 ± 1.03	2.86 ± 0.33	5.16 ± 0.2	11.96 ± 3.21
cyclin-dependent kinase 5	AA401479	2.13 ± 0.77	3.01 ± 0.69	11.06 ± 0.55	5.24 ± 1.28

Table 1. *cont.*

Gene name	Accession	A4	A24	S4	S24
cysteine knot superfamily 1, BMP antagonist 1	W47324	1.36 ± 0.42	2.2 ± 0.17	7.12 ± 2.17	8.99 ± 2.15
cysteine-rich, angiogenic inducer, 61	AI014487	5.7 ± 0.88	2.53 ± 0.2	7.55 ± 1.09	14.32 ± 6.17
endothelin receptor type A	AA450009	2.82 ± 1.71	3.15 ± 0.66	17.85 ± 10.98	11.95 ± 6.19
follistatin	AI214697	3.03 ± 2.16	5.34 ± 2.07	6.02 ± 4.83	48.46 ± 16.23
GTP binding protein overexpressed in skeletal muscle	AA418077	0.74 ± 0.03	4.92 ± 1.22	13.69 ± 11.06	77.57 ± 33.61
H3 histone, family 3B (H3.3B)	AA608514	2.94 ± 0.41	1.17 ± 0.03	3 ± 0.25	3.71 ± 0.58
hexabrachion (tenascin C, cytotactin)	AW075585	1.38 ± 0.62	3.11 ± 0.61	6.7 ± 3.44	9.63 ± 4.8
keratin 17	AA159201	1.02 ± 0.04	3.69 ± 1.11	7.55 ± 4.64	1.88 ± 0.33
keratin 19	AA464250	1.19 ± 0.26	3.67 ± 0.48	2.34 ± 0.28	4.53 ± 1.72
laminin, alpha 4	R43734	3.23 ± 0.19	0.88 ± 0.18	2.18 ± 0.12	3.94 ± 0.05
matrix metalloproteinase 1 (interstitial collagenase)	AA143201	3 ± 1.55	7.35 ± 2.2	1.48 ± 0.53	2.57 ± 0.31
matrix metalloproteinase 12 (macrophage elastase)	R92994	3.31 ± 1.13	4.66 ± 1.5	3.58 ± 0.69	5.31 ± 1.71
matrix metalloproteinase 17 (membrane-inserted)	R17506	2.04 ± 0.58	3.85 ± 1.02	5.1 ± 2.9	5.37 ± 2.53
milk fat globule-EGF factor 8 protein	AA054753	4.05 ± 0.41	0.69 ± 0.07	2.74 ± 1.35	1.97 ± 0.36
proline rich 2	AA669637	4.09 ± 1.25	1.08 ± 0.29	– ± –	3.65 ± 1.61
proteoglycan 1, secretory granule	AA278759	2.78 ± 0.31	3.75 ± 0.32	4.31 ± 0.95	11.02 ± 1.13
putative lymphocyte G0/G1 switch gene	AA931758	1.51 ± 0.32	5.86 ± 0.68	4.16 ± 1.47	19.65 ± 4.78
ret proto-oncogene	H24956	4.65 ± 1.63	2.08 ± 0.11	5.51 ± 3.09	3.32 ±1.68
Similar to serine (or cysteine) proteinase inhibitor, clade E member 2,	N57754	1.01 ± 0.16	4.2 ± 0.39	0.81 ± 0.07	9.26 ± 1.92
stanniocalcin 1	AA126561	2.43 ± 1.57	6.07 ± 1.35	– ± –	28.57 ± 11.95
tetraspan 1	AI674349	2.09 ± 0.92	3.12 ± 0.34	1.63 ± 1.09	1.15 ± 0.07
thrombospondin 1	AA007557	1.87 ± 0.33	4.29 ± 1.38	4.48 ± 0.48	9.22 ± 0.43
thyroid hormone receptor (v-erb-a) oncogene homologue, avian)	R60006	3.68 ± 1.04	1.49 ± 0.19	4.91 ± 1.12	3.33 ± 1.3
tissue factor pathway inhibitor 2	AA293402	3.45 ± 1.66	4.15 ± 0.47	3.88 ± 3.48	17.03 ± 8.93
tissue inhibitor of metalloproteinase 1	AA284534	0.98 ± 0.08	4.1 ± 0.66	7.86 ± 0.59	45.95 ± 14.06
transforming growth factor, beta-induced, 68 kDa	AA633901	1.54 ± 0.48	2.85 ± 0.41	1.06 ± 0.46	6.32 ± 1.31
Signalling molecules/Transcription factors					
jun B proto-oncogene	AA454711	5.67 ± 0.71	2.42 ± 0.27	9.84 ± 1.82	6.63 ± 1.94
jun D proto-oncogene	AA418670	2.51 ± 0.36	0.71 ± 0.08	2.67 ± 0.94	0.99 ± 0.36
mitogen activated protein kinase kinase 3	AA829383	3.62 ± 1.78	1.3 ± 0.37	1.98 ± 0.41	5.02 ± 1.91
nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	W55872	5.28 ± 0.27	1.21 ± 0.27	5.73 ± 2.29	4.57 ± 0.47
pellino homologue 1 (Drosophila)	H72755	5.13 ± 3.17	0.88 ± 0.09	0.45 ± 0.04	2.15 ± 0.09
protein phosphatase 2 (formerly 2 A), catalytic subunit, beta isoform	AA490473	1.71 ± 0.31	0.84 ± 0.15	2.84 ± 1.14	1.09 ± 0.53
protein tyrosine phosphatase type IVA, member 1	AA482193	2.12 ± 0.89	1.25 ± 0.14	2.51 ± 0.37	1.3 ± 0.28
retinoic acid induced 3	AA172400	4.71 ± 0.44	2.68 ± 0.12	3.79 ± 0.55	1.93 ± 1.11
sequestosome 1	AW074995	3.52 ± 0.95	1.06 ± 0.22	3.55 ± 1.36	3.8 ± 1.13
STAT induced STAT inhibitor 3	AI922872	11.5 ± 2.49	6.02 ± 1.94	2.92 ± 0.44	7.38 ± 2.59
transcription elongation factor B (SIII), polypeptide 3 (110 kDa, elongin A)	AA128607	5.52 ± 1.45	4.48 ± 0.83	17.5 ± 7.19	3.48 ± 1.47
translation factor sui1 homologue	AA488391	2.15 ± 0.15	1.57 ± 0.24	3.14 ± 0.9	2.53 ± 0.43
v-fos FBJ murine osteosarcoma viral oncogene homologue	R12840	9.58 ± 1.31	3.95 ± 1.04	7.6 ± 3.85	5.07 ± 0.91
v-jun sarcoma virus 17 oncogene homologue (avian)	AA293362	2.72 ± 0.44	0.87 ± 0.23	5.22 ± 1.26	2.5 ± 0.06
v-myc myelocytomatosis viral oncogene homologue (avian)	W87741	3.76 ± 0.78	2.69 ± 0.87	5.14 ± 0.26	4.11 ± 1.52
zinc finger protein 36, C3H type, homologue (mouse)	R38383	4.34 ± 1.26	1.61 ± 0.32	5.11 ± 0.97	3.02 ± 0.35
Metabolic enzymes					
6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3	N64010	12.14 ± 1.79	3.77 ± 0.75	8.98 ± 7.53	5.73 ± 1.94
alanine-glyoxylate aminotransferase	N57872	10.18 ± 1.1	3.59 ± 0.22	29.84 ± 11.45	16.33 ± 2.42
enolase 1 (alpha)	AI001174	0.97 ± 0.11	2.43 ± 0.36	1.56 ± 0.38	2.61 ± 0.56
enolase 2 (gamma, neuronal)	AI969131	1.9 ± 0.11	5.01 ± 2.04	1.28 ± 0.36	8.35 ± 3.92
EST, Highly similar to I39435 fructose-bisphosphate aldolase [H.sapiens]	AA977896	3.17 ± 0.06	2.58 ± 0.55	6.26 ± 1.65	22.61 ± 15.18
glyceraldehyde-3-phosphate dehydrogenase	AA197335	0.84 ± 0.21	3 ± 0.31	3.55 ± 3.01	2.37 ± 1.11
lactate dehydrogenase A	AA489611	1.48 ± 0.07	2.03 ± 0.19	1.88 ± 0.38	2.77 ± 0.93
ornithine decarboxylase 1	AA460115	2.54 ± 0.41	1.86 ± 0.61	1.61 ± 0.56	2.29 ± 0.11
UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyl-transferase 6	AI300634	9.72 ± 5.25	5.01 ± 1.82	14.98 ± 3.81	25.53 ± 8.79
Clotting/haemorrhage					
haemoglobin, alpha 2	AA027832	0.86 ± 0.11	5.14 ± 2.01	26.86 ± 22.75	15.63 ± 5.69
haemoglobin, beta	AI927438	0.94 ± 0.14	4.45 ± 0.85	4.89 ± 1.45	3.69 ± 0.79
haemoglobin, gamma A	H94956	1.1 ± 0.64	4.3 ± 1.66	1.75 ± 0.45	0.86 ± 0.04
haemoglobin, gamma G	N68719	1.31 ± 0.04	4.31 ± 0.61	4.45 ± 1.24	5.43 ± 0.39
plasminogen activator, tissue	AA447797	2.56 ± 0.91	3.02 ± 0.37	6.51 ± 2.9	3.04 ± 1.03
plasminogen activator, urokinase	AA284668	3.87 ± 1.46	3.07 ± 0.06	9.55 ± 2.31	25.36 ± 21.25

Table 1. *cont.*

Gene name	Accession	A4	A24	S4	S24
Motility and adherence					
carcinoembryonic antigen-related cell adhesion molecule 5	AA055605	1.29 ± 0.51	2.91 ± 0.01	0.97 ± 0.39	4.29 ± 2.2
coronin, actin binding protein, 1 A	AA047477	0.78 ± 0.03	2.82 ± 0.45	1.71 ± 0.36	3.69 ± 0.54
filamin (human actin binding protein C)	T64982	2.46 ± 0.96	2.21 ± 0.12	2.96 ± 1.79	5.9 ± 4.5
homologous to supervillin	T57810	4.59 ± 1.18	3.24 ± 0.69	9.35 ± 2.65	8.94 ± 1.6
intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	N68859	4 ± 1.05	3.12 ± 0.35	13.83 ± 5.21	10.29 ± 3.38
plectin 1, intermediate filament binding protein, 500 kDa	AA448400	2.23 ± 0.41	1.07 ± 0.2	2.96 ± 0.78	1.48 ± 0.92
Transporters					
solute carrier family 15 (H^+/peptide transporter), member 2	AA425352	4.23 ± 1.68	2.93 ± 0.18	23.9 ± 13.56	6.15 ± 0.61
solute carrier family 2 (facilitated glucose transporter), member 1	R11688	6.43 ± 1.99	7.31 ± 1.26	5.17 ± 3.17	24.33 ± 16.79
solute carrier family 16 (monocaborxylic acid transporters), member 3	AA129777	3.89 ± 0.79	3.05 ± 0.49	2.88 ± 0.63	7.70 ± 1.24
Unknown					
AA121141	AA121141	1.44 ± 0.22	2.55 ± 0.33	2.28 ± 0.35	7.05 ± 2.24
AA128925	AA128925	3.9 ± 0.68	2.25 ± 0.26	6.05 ± 0.94	3.22 ± 0.93
chromosome 8 open reading frame 4	H16793	3.04 ± 0.57	4.43 ± 0.67	4.01 ± 0.73	9.95 ± 4.62
erythrocyte membrane protein band 7.2 (stomatin)	R62817	1.59 ± 0.42	2.52 ± 0.52	0.7 ± 0.27	7.34 ± 4.5
ESTs, Weakly similar to T28770 hypothetical protein W03D2.1	AI003775	3.91 ± 1	2.68 ± 0.53	11.1 ± 3.94	17.7 ± 5.48
H73321	H73321	4.08 ± 2.02	2.59 ± 0.28	10.86 ± 3	23.17 ± 10.11
Homo sapiens mRNA; cDNA DKFZp564C2063	AA135912	4.3 ± 1.04	0.93 ± 0.24	6.07 ± 2.78	4.98 ± 1.71
Homo sapiens, clone MGC:12617 IMAGE:2964706, mRNA, complete cds	H71824	8.46 ± 3.71	10.44 ± 7.01	5.94 ± 1.25	7.99 ± 1.42
homologous to yeast nitrogen permease (candidate tumor suppressor)	AI222722	2.56 ± 0.82	2.14 ± 0.66	7.36 ± 0.92	4.71 ± 0.09
Human mRNA for ZFM1 protein alternatively spliced product	AI356319	1.38 ± 0.52	5.42 ± 2.97	6.77 ± 2.91	2.85 ± 0.46
hypothetical protein FLJ23138	H26181	3.3 ± 1.15	2.31 ± 0.01	4.56 ± 0.16	3.09 ± 0.12
nuclear receptor subfamily 4, group A, member 1	N94487	3.01 ± 0.52	1.64 ± 0.14	10.73 ± 5.33	16.14 ± 10.58
Similar to RNA helicase-related protein, clone MGC:9246 IMAGE:3892441	AA872383	7.48 ± 2.35	2.83 ± 0.1	9.62 ± 0.63	9.38 ± 1.35
SUID310180		3.65 ± 1.56	4.1 ± 1.97	5.97 ± 0.54	8.96 ± 1.98
SUID310693		1.55 ± 0.5	2.6 ± 0.39	1.76 ± 0.03	15.89 ± 10.35
SUID310866		2.67 ± 0.35	3.57 ± 1.33	7.04 ± 4.03	4.02 ± 0.9

The gene name, accession number, and mean fold increase (over the values from uninfected human colonic xenografts) from the replicates ± the standard error of the mean are shown for each gene in *E. histolytica* infection at 4 h (A4), 24 h (A24), and *S. flexneri* infection at 4 h (S4) and 24 h (S24). When intensity values did not meet criteria to allow a mean to be obtained for a given gene at a given time-point, – ± – is shown. Genes were placed within categories based on known functions or the functions of homologues.

expression in shigella-infected human colonic xenografts, but did not meet the criteria for increased expression in amoeba-infected xenografts (Table 2, Fig. 3). Fifty-four of these genes were also represented on the Incyte array, and 44/54 (81%) were confirmed as showing a similar expression pattern to that detected in the Stanford arrays. Many of the genes in this group were expressed at high levels in *S. flexneri* infection at 4 h, but not at the other time-points (Fig. 3). The largest grouping among shigella-specific genes was those genes encoding proteins that are involved in cytoskeletal rearrangements or cell motility (Table 2). The genes encoding the intermediate filament proteins, vimentin and desmin, the microtubule-related protein dynein, the actin-interacting proteins caldesmon, smoothelin, transgelin (TAGLN), gelsolin, moesin, ponsin, myosin, tropomyosin, as well as several isoforms of actin itself, all showed higher expression in human colonic xenografts infected with *S. flexneri*. The increased expression of genes encoding a group of proteins that may regulate cytokine signalling (elongin C, SWIP-1, and the WD-repeat protein) (Kamura *et al*., 1998) was also detected in *S. flexneri*-infected human colonic xenografts. Several genes encoding proteins linked to the regulation of cellular proliferation or apoptosis were identified as shigella-specific, including galectin 1, programmed cell-death 5, BTG family member 2, thymosin β-10, and ubiquinone. Three members of the large RGS family (regulators of G-protein-signalling) were also specifically increased in *S. flexneri* infection. Two chemokines, eotaxin and tyrosyl-t-RNA synthetase, were up-regulated in *S. flexneri* infection, but not in *E. histolytica*-infected colon (Table 2).

Genes showing increased expression in E. histolytica *infection, but not* S. flexneri *infection*

Only 39 genes that met criteria for increased expression in *E. histolytica*-infected human colonic xenografts but not in *S. flexneri*-infected xenografts were found (Table 3, Fig. 4). Twenty-seven of these genes were also

Table 2. Genes showing increased expression in *S. flexneri* infection but not *E. histolytica* infection at one or both time-points.

Gene name	Accession	A4	A24	S4	S24
Stress response					
amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)	AA128501	0.75 ± 0.09	1.56 ± 0.34	1.64 ± 0.36	3.89 ± 1.45
heat shock 90 kDa protein 1, beta	AW075411	1.16 ± 0.23	1.35 ± 0.13	1.57 ± 0.49	2.64 ± 0.8
Similar to heat shock protein, 30 kDa, clone MGC:17057 IMAGE:4345594	AA064917	1.11 ± 0.2	1.05 ± 0.16	2.03 ± 0.83	3.76 ± 0.94
NADH dehydrogenase (ubiquinone)	AA214053	0.77 ± 0.23	0.78 ± 0.04	2.28 ± 0.27	1.62 ± 0.57
Immune response/Inflammatory mediators/Cytokines/Chemokines					
complement component 1, s subcomponent	T62048	1.27 ± 0.19	1.11 ± 0.3	2.42 ± 0.31	3.04 ± 1.04
interferon induced transmembrane protein 1 (9–27)	AA058323	1.04 ± 0.41	1.21 ± 0.26	6.27 ± 2.8	3.6 ± 1.41
interferon induced transmembrane protein 2 (1–8D)	AA985421	1.17 ± 0.12	1.13 ± 0.21	3.58 ± 0.64	3.93 ± 1.35
small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin)	W69211	0.72 ± 0.2	1.09 ± 0.23	57.71 ± 50.53	2.63 ± 1.12
SOCS box-containing WD protein SWiP-1	R27345	0.95 ± 0.27	1.23 ± 0.12	2.08 ± 0.67	2.01 ± 0.06
tyrosyl-tRNA synthetase	AA486761	1.26 ± 0.24	0.71 ± 0.14	2.32 ± 0.36	2.98 ± 1.04
WD-repeat protein	AI935306	0.96 ± 0.3	0.38 ± 0.09	5.63 ± 0.34	1.28 ± 0.4
Growth Factors/Tissue Remodelling/Cell Cycle Regulators/Apoptosis-related					
annexin A1	H63077	1.11 ± 0.22	1 ± 0.05	3.18 ± 0.98	2.89 ± 0.1
ATP-binding cassette, subfamily C (CFTR/MRP), member 1	AA424804	1.72 ± 0.35	1.55 ± 0.18	3.91 ± 1.48	3.98 ± 1.56
BTG family, member 2	H69582	1.84 ± 0.63	1.36 ± 0.34	4.44 ± 2.9	17.85 ± 15.56
cargo sorting protein – mannose 6 phosphate receptor binding protein	AA416787	1.87 ± 0.49	1.15 ± 0.23	4.79 ± 1.82	1.4 ± 0.59
CD53 antigen	H70124	2.16 ± 0.33	1.72 ± 0.43	11.88 ± 3.48	15.62 ± 9.52
collagen, type I, alpha 1	R48843	0.68 ± 0.18	0.41 ± 0.11	2.34 ± 0.43	0.45 ± 0.13
collagen, type I, alpha 2	AA490172	0.91 ± 0.22	0.38 ± 0.02	2.06 ± 0.31	0.38 ± 0.15
collagen, type IV, alpha 1	R78225	0.49 ± 0.12	0.97 ± 0.19	4.6 ± 1.12	5.64 ± 0.89
collagen, type IV, alpha 2	AA430540	1.11 ± 0.43	1.5 ± 0.36	1.85 ± 0.48	2.68 ± 0.68
collagen, type V, alpha 2	AA461456	2.46 ± 0.76	0.54 ± 0.04	3.92 ± 1.74	1.26 ± 0.73
collagen, type VI, alpha 3	R62603	0.87 ± 0.05	0.78 ± 0.09	3.01 ± 0.48	1.78 ± 0.19
C-type lectin, superfamily member 2 (activation-induced)	AA417921	0.89 ± 0.23	1.18 ± 0.21	3.61 ± 0.73	5.57 ± 0.89
cysteine and glycine-rich protein 1	AI935290	0.76 ± 0.12	0.62 ± 0.07	5.16 ± 0.5	1.51 ± 0.54
early development regulator 2 (polyhomeotic 2 homologue)	AA598840	1.07 ± 0.41	1.01 ± 0.14	1.67 ± 0.1	3.85 ± 1.82
fatty acid binding protein 4, adipocyte	AI652163	1.28 ± 0.63	0.62 ± 0	5.01 ± 0.9	2.84 ± 0.5
fibroblast growth factor 7 (keratinocyte growth factor)	AA009608	1.55 ± 0.81	2.33 ± 0.74	2.68 ± 1.99	7.14 ± 5.4
fibroblast growth factor receptor 1	R54610	1.27 ± 0.05	0.91 ± 0.14	1.33 ± 0.52	2.86 ± 0.26
Homo sapiens COAS3 (COAS3) mRNA, complete cds	AA018229	0.78 ± 0.06	1.72 ± 0.47	1.96 ± 0.05	1.74 ± 0.44
insulin-like growth factor binding protein 7	T53297	1 ± 0.26	0.64 ± 0.19	4.58 ± 1.63	2.7 ± 0.06
lectin, galactoside-binding, soluble, 1 (galectin 1)	AI927284	0.83 ± 0.1	0.69 ± 0.08	3.51 ± 0.47	1.94 ± 0.11
matrix Gla protein	AA155913	0.68 ± 0.1	0.94 ± 0.16	6.93 ± 0.8	2.46 ± 0.15
programmed cell death 5	AA416757	1.11 ± 0.22	1.32 ± 0.27	2.28 ± 0.61	2.02 ± 0.61
regenerating islet-derived 1 alpha	AA625655	0.9 ± 0.14	0.78 ± 0.03	1.25 ± 0.2	2.77 ± 1.01
Sec61 gamma	W96106	1.46 ± 0.53	1.75 ± 0.38	2.19 ± 0.1	3.88 ± 1.49
serine (or cysteine) proteinase inhibitor, clade H	R71093	0.77 ± 0.27	0.86 ± 0.1	1.49 ± 0.71	5.7 ± 2.59
sorting nexin 14	AA524965	1.18 ± 0.42	0.95 ± 0.26	– ± –	4.64 ± 1.45
SPARC-like 1 (mast9, hevin)	AA490471	1.18 ± 0.43	0.57 ± 0.16	2.05 ± 0.32	4.61 ± 4.27
TGFB-induced factor (TALE family homeobox)	H51705	1.98 ± 0.71	1.65 ± 0.49	1.43 ± 0.59	3.98 ± 0.25
thymosin, beta 10	AA486085	0.89 ± 0.27	1.63 ± 0.2	2.46 ± 0.32	2.52 ± 0.46
transcription elongation factor B (SIII), polypeptide 1 (15 kDa, elongin C)	W81684	1.85 ± 0.96	0.87 ± 0.03	1.95 ± 0.58	3.24 ± 0.43
Signalling Molecules/Transcription Factors					
AE binding protein 1	AA490462	1.77 ± 0.24	1.19 ± 0.19	3.16 ± 0.42	2.61 ± 0.21
basic transcription element binding protein 1	T63779	1.26 ± 0.19	1.02 ± 0.06	2.65 ± 1.03	2.29 ± 0.35
EphA5 (ephrin A5)	H11658	1.77 ± 0.35	1.31 ± 0.44	3.34 ± 0.54	1.86 ± 0.51
eukaryotic translation initiation factor 4 A, isoform 1	AA650175	1.86 ± 0.27	1.47 ± 0.61	2.09 ± 0.35	2.74 ± 0.18
regulator of G-protein signalling 1	AA017417	0.83 ± 0.25	0.9 ± 0.11	7.61 ± 6.69	2.66 ± 0.98
regulator of G-protein signalling 16	AA453774	– ± –	2.19 ± 1.44	8.16 ± 3.47	5.11 ± 3.73
regulator of G-protein signalling 2, 24 kDa	AI675670	1.53 ± 0.29	1.11 ± 0.08	2.51 ± 0.43	2.15 ± 0.64
Metabolic enzymes					
aldolase A, fructose-bisphosphate	AA775241	1.82 ± 0.27	1.69 ± 0.11	1.62 ± 0.64	2.62 ± 0.67
phosphoglycerate mutase 1 (brain)	AW071046	0.92 ± 0.22	1.03 ± 0.13	1.48 ± 0.31	3.32 ± 1.28
steroid-5-alpha-reductase, alpha polypeptide 1	H16833	1.02 ± 0.33	1.84 ± 0.35	1.49 ± 0.64	17.56 ± 7.1
Motility/Adherence/Cytoskeletal proteins					
actin, alpha 2, smooth muscle, aorta	AA634006	1.04 ± 0.16	0.54 ± 0.06	5.78 ± 3.94	1.61 ± 0.08
actin, gamma 2, smooth muscle, enteric	T60048	0.51 ± 0.12	0.56 ± 0.08	3.83 ± 0.49	1.36 ± 0.57
caldesmon 1	H51958	0.32 ± 0.13	0.73 ± 0.23	3.6 ± 0.76	1.48 ± 0.36
desmin	AI972874	0.73 ± 0.33	0.57 ± 0.12	3.41 ± 1.38	1.01 ± 0.56
dynein, cytoplasmic, heavy polypeptide 1	AA010589	0.95 ± 0.34	0.62 ± 0.2	5.25 ± 1.41	1.85 ± 0.64
fibronectin 1	AI262682	0.87 ± 0.31	0.64 ± 0.1	2.44 ± 0.12	1.23 ± 0.37

Table 2. *cont.*

Gene name	Accession	A4	A24	S4	S24
gelsolin (amyloidosis, Finnish type)	H72027	1.79 ± 0.58	0.72 ± 0.08	4.77 ± 1.2	1.01 ± 0.31
lumican	AA447781	0.94 ± 0.25	0.31 ± 0.12	2.73 ± 0.52	0.42 ± 0.22
moesin	R22977	1.58 ± 1.2	1.22 ± 0.11	10.12 ± 6.58	5.66 ± 1.28
myosin, heavy polypeptide 11, smooth muscle	AA126989	0.43 ± 0.25	0.46 ± 0.07	2.47 ± 0.31	0.26 ± 0.11
myosin, light polypeptide 9, regulatory	AA877166	0.63 ± 0.28	0.59 ± 0.23	2.39 ± 0.49	0.99 ± 0.02
myosin, light polypeptide kinase	AA487215	1.18 ± 0.52	0.75 ± 0.08	1.89 ± 0.22	0.93 ± 0.1
SH3-domain protein 5 (ponsin)	AA459944	1.6 ± 0.17	1.21 ± 0.27	4.34 ± 0.46	0.96 ± 0.38
smoothelin	AA449234	1.05 ± 0.3	0.62 ± 0.12	3.75 ± 0.26	1.35 ± 0.64
transgelin	AA010664	1.06 ± 0.4	0.55 ± 0.18	4.95 ± 1.82	2.15 ± 1.05
tropomyosin 1 (alpha)	AA047403	1.22 ± 0.28	0.42 ± 0.29	3.88 ± 0.52	1.86 ± 0.94
tubulin, alpha 1 (testis specific)	AA180742	5.67 ± 4.7	1 ± 0.07	1.81 ± 0.33	2.04 ± 0.67
vimentin	AA147847	0.92 ± 0.2	0.78 ± 0.1	3.19 ± 0.46	1.73 ± 0.57
vinculin	AA486727	1.85 ± 1.1	0.87 ± 0.11	7.64 ± 5.23	5.44 ± 2.64
actin related protein 2/3 complex, subunit 1B (41 kDa)		1.23 ± 0.25	1.40 ± 0.07	2.87 ± 0.58	2.78 ± 0.85
Unknown					
AA035657	AA035657	0.84 ± 0.18	0.23 ± 0.09	2.27 ± 0.12	0.3 ± 0.1
AA402898	AA402898	0.21 ± 0.04	0.73 ± 0.29	3.32 ± 0.05	2.86 ± 1.46
AA573736	AA573736	1.3 ± 0.48	1.05 ± 0.24	3.6 ± 0.31	2.58 ± 0.69
Homo sapiens cDNA FLJ20767 fis, clone COL06986	AA043349	0.85 ± 0.26	0.76 ± 0.14	6.89 ± 1.48	0.92 ± 0.47
Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021)	AW005791	1.51 ± 0.28	0.57 ± 0.05	3.32 ± 0.56	0.93 ± 0.23
hypothetical protein MGC3036	AA468776	0.6 ± 0.26	0.46 ± 0.04	7.74 ± 2.52	3.96 ± 2.2
KIAA0171 gene product	AI935584	1.01 ± 0.09	0.54 ± 0.13	2.2 ± 0.3	1.01 ± 0.22
MSTP032 protein	W60845	0.68 ± 0.14	0.35 ± 0.02	4.04 ± 0.75	1.88 ± 0.85
T64570	T64570	0.84 ± 0.09	0.98 ± 0.24	3.37 ± 1.31	2.01 ± 0.11
zinc finger protein 134 (clone pHZ-15)	AA976255	0.67 ± 0.21	0.5 ± 0.1	3.72 ± 0.62	1.26 ± 0.38

The gene name, accession number, and mean fold increase (over the values from uninfected human colonic xenografts) from the replicates ± the standard error of the mean are shown for each gene in *E. histolytica* infection at 4 h (A4), 24 h (A24), and *S. flexneri* infection at 4 h (S4) and 24 h (S24). When intensity values did not meet criteria to allow a mean to be obtained for a given gene at a given time-point, – ± – is shown. Genes were placed within categories based on known functions or the functions of homologues.

represented on the Incyte array, and 21/27 (78%) showed a similar expression pattern in the confirmatory experiments. Many of the genes in this group were expressed at higher levels in *E. histolytica* infection at 4 h, but not at other time points (Fig. 4). Among the largest groupings within the amoeba-specific genes were genes that encode proteins involved in the junctions between cells (claudin 4, catenin, junction plakoglobin), and proteins implicated in anchoring cells to the extracellular matrix (integrins alpha 6, integrin beta 4, integrin beta 4 binding protein, laminin gamma 2, laminin beta 3) (Table 3). Four distinct serine protease genes showed increased expression only in *E. histolytica* infected human colonic xenografts, while both chains of the interferon γ receptor were activated in *E. histolytica* infection. Several genes showed increased expression in *E. histolytica* and decreased expression in *S. flexneri*, including alpha defensin 3 (Fig. 4).

Confirmation of differential gene expression by Real-time PCR

In these experiments additional SCID-HU-INT mice were infected with *S. flexneri* or *E. histolytica* under the identical conditions used for the microarray studies, and RNA was obtained at 4 or 24 h after infection for analysis of gene expression by Real-time PCR. We looked for changes in expression levels for a total of 17 selected genes that had demonstrated increased expression by microarray analysis in *E. histolytica* infection alone (α-defensin, trypsin 2, claudin 4, laminin γ2, and serine 2 transmembrane protease), in *S. flexneri* infection alone (vimentin, vinculin, insulin-like growth factor binding protein 7, cysteine and glycine-rich protein 1, eotaxin, and hypothetical protein MGC3036), or in both *S. flexneri* and *E. histolytica* infection (junB, metallothionein 1 L, GRO1 oncogene, interleukin-6, and interleukin-11). As shown in Fig. 5, Real-time PCR confirmed the microarray findings of increased expression of junB, metallothionein 1 L, GRO1, IL-6, and IL-11 in both *E. histolytica* and *S. flexneri* infection. The pattern of expression for each of the genes was generally similar for the microarray and Real-time PCR results, with the exception of IL-11, which appeared to specifically be increased late in shigella or *E. histolytica* infection by microarray analysis, but showed increased levels at both 4 and 24 h in the Real-time PCR analysis. Similarly, genes identified as specifically increased in *S. flexneri* infection of human colonic xenografts by the microarray analysis were confirmed as shigella-specific by Real-time PCR, as was their general pattern of expression (highest at *S. flexneri* infection at 4 h) (Fig. 5C). Real-time PCR analysis also confirmed that the genes identified as specifically increased in *E. histolytica* infection by the microarray

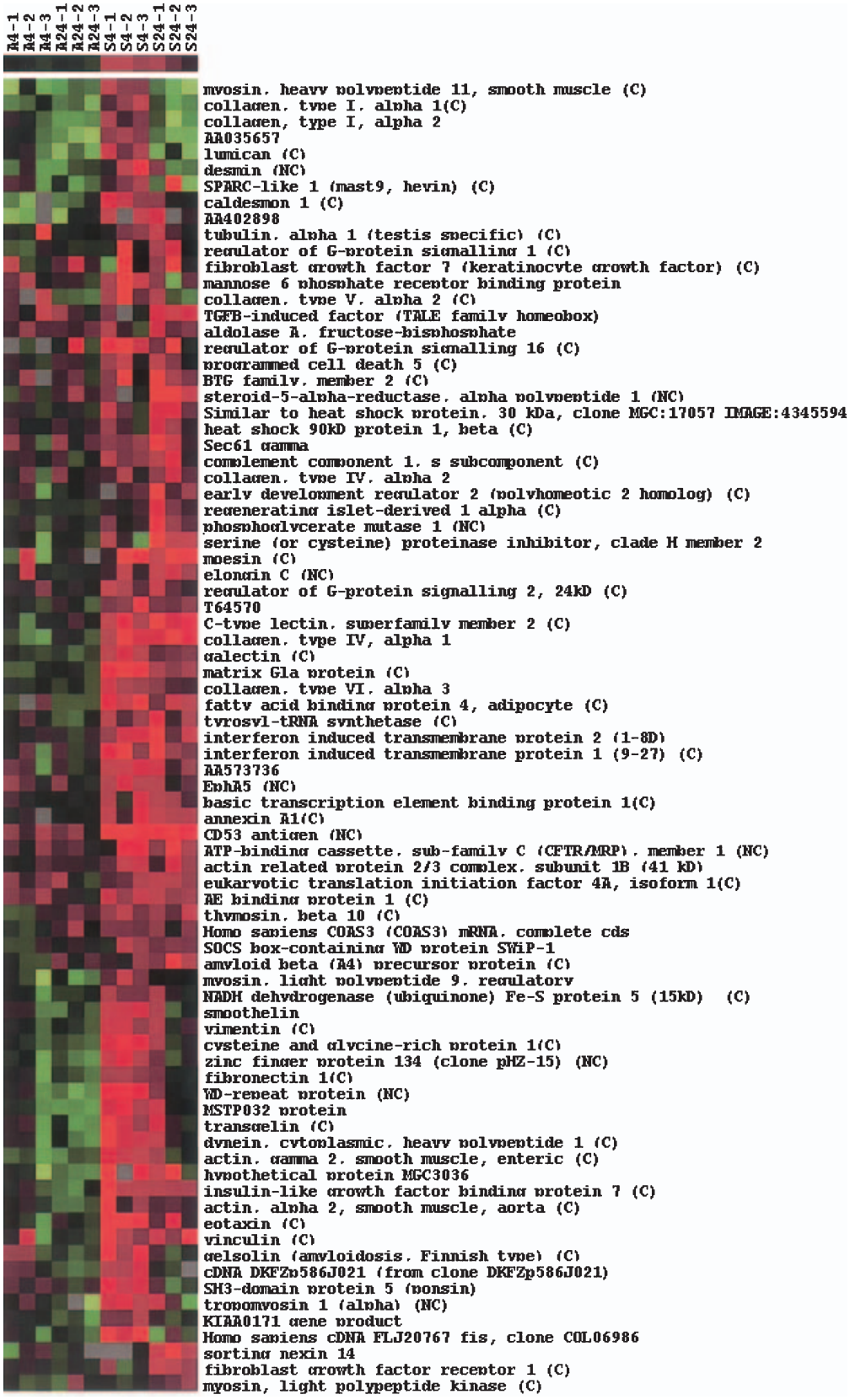
A4-1
A4-2
A4-3
A24-1
A24-2
A24-3
S4-1
S4-2
S4-3
S24-1
S24-2
S24-3
myosin, heavy polypeptide 11, smooth muscle (C)
collagen, type I, alpha 1(C)
collagen, type I, alpha 2
AA035657
lumican (C)
desmin (NC)
SPARC-like 1 (mast9, hevin) (C)
caldesmon 1 (C)
AA402898
tubulin, alpha 1 (testis specific) (C)
regulator of G-protein signalling 1 (C)
fibroblast growth factor 7 (keratinocyte growth factor) (C)
mannose 6 phosphate receptor binding protein
collagen, type V, alpha 2 (C)
TGFB-induced factor (TALE family homeobox)
aldolase A, fructose-bisphosphate
regulator of G-protein signalling 16 (C)
programmed cell death 5 (C)
BTG family, member 2 (C)
steroid-5-alpha-reductase, alpha polypeptide 1 (NC)
Similar to heat shock protein, 30 kDa, clone MGC:17057 IMAGE:4345594
heat shock 90kD protein 1, beta (C)
Sec61 gamma
complement component 1, s subcomponent (C)
collagen, type IV, alpha 2
early development regulator 2 (polyhomeotic 2 homolog) (C)
regenerating islet-derived 1 alpha (C)
phosphoglycerate mutase 1 (NC)
serine (or cysteine) proteinase inhibitor, clade H member 2
moesin (C)
elongin C (NC)
regulator of G-protein signalling 2, 24kD (C)
T64570
C-type lectin, superfamily member 2 (C)
collagen, type IV, alpha 1
galectin (C)
matrix Gla protein (C)
collagen, type VI, alpha 3
fatty acid binding protein 4, adipocyte (C)
tyrosyl-tRNA synthetase (C)
interferon induced transmembrane protein 2 (1-8D)
interferon induced transmembrane protein 1 (9-27) (C)
AA573736
EphA5 (NC)
basic transcription element binding protein 1(C)
annexin A1(C)
CD53 antigen (NC)
ATP-binding cassette, sub-family C (CFTR/MRP), member 1 (NC)
actin related protein 2/3 complex, subunit 1B (41 kD)
eukaryotic translation initiation factor 4A, isoform 1(C)
AE binding protein 1 (C)
thymosin, beta 10 (C)
Homo sapiens COAS3 (COAS3) mRNA, complete cds
SOCS box-containing WD protein SWiP-1
amyloid beta (A4) precursor protein (C)
myosin, light polypeptide 9, regulatory
NADH dehydrogenase (ubiquinone) Fe-S protein 5 (15kD) (C)
smoothelin
vimentin (C)
cysteine and glycine-rich protein 1(C)
zinc finger protein 134 (clone pHZ-15) (NC)
fibronectin 1(C)
WD-repeat protein (NC)
MSTP032 protein
transgelin (C)
dynein, cytoplasmic, heavy polypeptide 1 (C)
actin, gamma 2, smooth muscle, enteric (C)
hypothetical protein MGC3036
insulin-like growth factor binding protein 7 (C)
actin, alpha 2, smooth muscle, aorta (C)
eotaxin (C)
vinculin (C)
gelsolin (amyloidosis, Finnish type) (C)
cDNA DKFZp586J021 (from clone DKFZp586J021)
SH3-domain protein 5 (bonsin)
tropomyosin 1 (alpha) (NC)
KIAA0171 gene product
Homo sapiens cDNA FLJ20767 fis, clone COL06986
sorting nexin 14
fibroblast growth factor receptor 1 (C)
myosin, light polypeptide kinase (C)

Table 3. Genes showing increased expression in *E. histolytica* infection but not *S. flexneri* infection at one or both time-points.

Gene Name	Accession	A4	A24	S4	S24
Stress response					
cytochrome P450, subfamily IIS, polypeptide 1	AA971278	5.32 ± 2.96	0.92 ± 0.12	0.92 ± 0.57	1.58 ± 0.84
glucagon	AI955772	0.72 ± 0.03	3.1 ± 0.2	0.16 ± 0.03	0.54 ± 0.13
cDNA FLJ31317 fis, moderately similar to CYTOCHROME P450 3A5	AA702706	2.33 ± 0.16	1.06 ± 0.21	0.85 ± 0.31	0.27 ± 0.1
Immune response/Inflammatory mediators/Cytokines/Chemokines					
interferon gamma receptor 1	H11482	6.4 ± 3.96	1.09 ± 0.39	0.47 ± 0.33	0.81 ± 0.42
defensin, alpha 3, neutrophil-specific	T81340	2.96 ± 0.24	0.93 ± 0.19	0.33 ± 0.12	0.22 ± 0.11
interferon gamma receptor 2 (interferon gamma transducer 1)	AA969475	2.86 ± 0.69	1 ± 0.18	1.47 ± 0.46	1.49 ± 1.05
coagulation factor II (thrombin) receptor-like 1 (PAR2)	AA454652	2.92 ± 0.06	1.10 ± 0.2	0.48 ± 0.22	0.35 ± 0.09
Growth factors/Tissue remodelling/Cell cycle regulators/Apoptosis-related					
transmembrane 4 superfamily member 3 (tetraspanin family)	AA045698	4.98 ± 2.83	1.31 ± 0.15	0.97 ± 0.26	1.04 ± 0.15
collagen, type XVI, alpha 1	AA088202	2.28 ± 0.22	0.83 ± 0.24	1.25 ± 0.46	0.48 ± 0.17
cytokeratin 20	AA522447	3.7 ± 0.08	1.35 ± 0.6	1.56 ± 0.33	1.49 ± 0.74
serine protease inhibitor, Kazal type 4	AA534438	0.74 ± 0.29	2.29 ± 0.16	1.53 ± 0.61	0.47 ± 0.07
collagen, type VII, alpha 1	AA598507	8.2 ± 6.26	2.84 ± 0.23	1.51 ± 0.55	0.84 ± 0.3
catenin (cadherin-associated protein), beta 1 (88 kDa)	AA442092	2.67 ± 0.28	1.15 ± 0.28	1.01 ± 0.36	1.47 ± 0.48
SH3-domain GRB2-like endophilin B1	AA465147	1.66 ± 0.21	2.32 ± 0.24	1.35 ± 0.54	1.29 ± 0.11
fatty acid binding protein 6, ileal (gastrotropin)	AI311734	0.58 ± 0.23	4.43 ± 0.46	1.07 ± 0.04	0.28 ± 0.12
tissue inhibitor of metalloproteinase 3	AA478662	1.15 ± 0.36	33.48 ± 29.09	1.68 ± 0.05	2.12 ± 1.25
discoidin domain receptor family, member 1	H41900	3.59 ± 0.89	0.84 ± 0.25	1.28 ± 0.48	0.41 ± 0.12
Signalling molecules/Transcription factors					
E74-like factor 3 (ets domain transcription factor, epithelial-specific)	AI984236	3.76 ± 0.31	0.92 ± 0.07	0.74 ± 0.09	0.34 ± 0.15
phospholipase A2, group IIA (platelets, synovial fluid)	T61271	2.38 ± 0.04	1.46 ± 0.09	0.27 ± 0.03	0.44 ± 0.08
H.sapiens mRNA for rho GDP-dissociation Inhibitor 1	BC028333	2.22 ± 0.12	1.25 ± 0.4	0.84 ± 0.17	0.9 ± 0.34
Metabolic Enzymes/Mucins					
spermidine/spermine N1-acetyltransferase	AA010811	2.01 ± 0.21	0.81 ± 0.15	0.4 ± 0.13	0.81 ± 0.25
alkaline phosphatase, intestinal	AA190871	2.29 ± 0.26	0.84 ± 0.02	0.34 ± 0.02	0.23 ± 0.04
mucin 3B	AI623883	3.6 ± 0.5	1.23 ± 0.18	1.04 ± 0.27	0.36 ± 0.12
glucosaminyl (N-acetyl) transferase 3, mucin type	AI955582	5.85 ± 2.75	6.52 ± 2.8	0.83 ± 0.33	1.9 ± 1.03
Motilty/Adherence/Junctional proteins					
integrin, beta 4	AA076430	2.13 ± 0.42	2.23 ± 0.19	0.75 ± 0.62	0.33 ± 0.09
laminin, gamma 2 (nicein (100 kDa), kalinin (105 kDa), BM600 (100 kDa)	AA677534	3.97 ± 0.16	4.17 ± 1.63	1.53 ± 0.42	1.24 ± 0.34
laminin, beta 3 (nicein (125 kDa), kalinin (140 kDa), BM600 (125 kDa))	AW007267	4.15 ± 0.44	1.19 ± 0.07	1.29 ± 0.44	1.08 ± 0.42
claudin 4	AA863314	3.53 ± 0.33	1.19 ± 0.13	1.12 ± 0.33	0.78 ± 0.08
junction plakoglobin	R06417	2.42 ± 0.25	1.36 ± 0.33	1 ± 0.24	0.79 ± 0.08
integrin, alpha 6	R17993	2.49 ± 0.37	1.45 ± 0.44	1.63 ± 0.62	3.04 ± 1.38
integrin beta 4 binding protein	AI017019	2.41 ± 0.75	0.77 ± 0.09	0.69 ± 0.17	1.13 ± 0.5
Transporters/Proteases					
chloride intracellular channel 2	AI311800	0.2 ± 0.07	6.7 ± 3.05	0.61 ± 0.02	– ± –
transmembrane protease, serine 2	AA579186	7.56 ± 0.28	1.47 ± 0.39	1.17 ± 0.44	0.57 ± 0.15
protease, serine, 2 (trypsin 2)	AA394198	3.01 ± 0.1	0.98 ± 0.13	0.62 ± 0.17	0.3 ± 0.08
protease, serine, 4 (trypsin 4, brain)	AI308916	2.31 ± 0.12	1.15 ± 0.28	0.71 ± 0.09	0.46 ± 0.09
protease, serine, 8 (prostasin)	R25981	2.31 ± 0.1	0.89 ± 0.15	2.44 ± 1.78	1.35 ± 1.3
Unknown					
clone MGC:22924 IMAGE:4843302, mRNA, complete cds	AA570148	2.65 ± 0.47	0.82 ± 0.17	0.21 ± 0.1	0.12 ± 0.03
cyclin L ania-6a	AA465166	2.95 ± 0.88	1.14 ± 0.27	1.5 ± 0.19	1.86 ± 0.45
Homo sapiens cDNA FLJ31206 fis, clone KIDNE2003335	T49815	4.38 ± 1.56	1.31 ± 0.11	0.5 ± 0.13	0.44 ± 0.02

The gene name, accession number, and mean fold increase (over the values from uninfected human colonic xenografts) from the replicates ± the standard error of the mean are shown for each gene in *E. histolytica* infection at 4 h (A4), 24 h (A24), and *S. flexneri* infection at 4 h (S4) and 24 h (S24). When intensity values did not meet criteria to allow a mean to be obtained for a given gene at a given time-point, – ± – is shown. Genes were placed within categories based on known functions or the functions of homologues.

Fig. 3. Genes specifically expressed in *S. flexneri*-infected human colonic xenografts at 4 and/or 24 h of infection. Gene expression profiles from three experiments looking at *E. histolytica* infection at 4 h (A4), and three experiments each for *E. histolytica* infection at 24 h (A24) and *S. flexneri* infection at 4 h (S4) and 24 h (S24) are shown. Genes are listed by gene name, and those genes showing a similar expression pattern in the confirmatory experiments using the Unigene array are marked by a (C). Those genes that were represented on the Unigene array but did not exhibit the same expression pattern are marked as (NC). The data are expressed as the $\log_2$ of the expression ratio, and the key for intensity of expression is: 

Grey bars signify missing values secondary to failure of a given gene spot to meet minimal intensity requirements for that experiment.

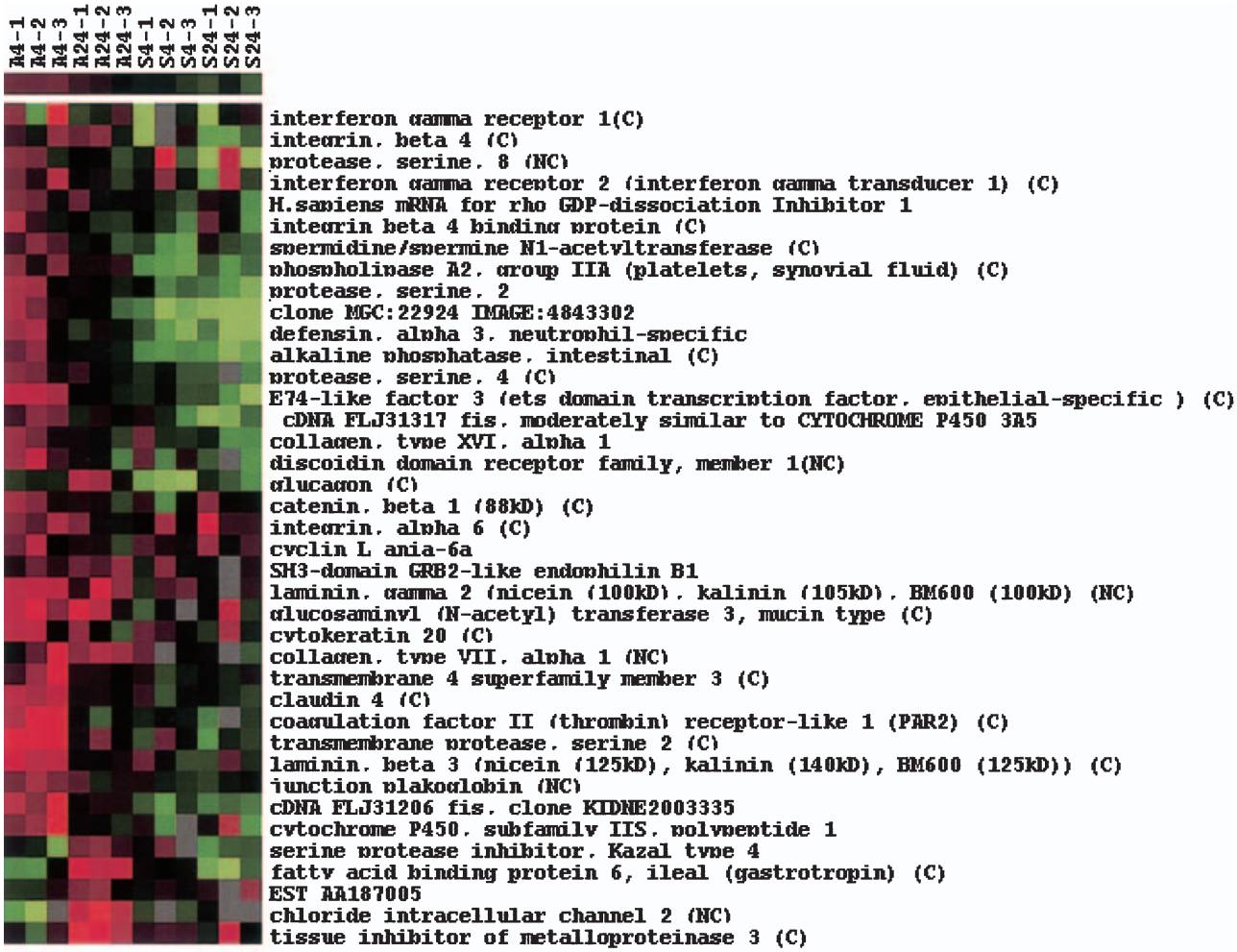

Fig. 4. Genes specifically expressed in *E. histolytica*-infected human colonic xenografts at 4 or 24 h of expression. Gene expression profiles from three experiments looking at *E. histolytica* infection at 4 h (A4), and three experiments each for *E. histolytica* infection at 24 h (A24) and *S. flexneri* infection at 4 h (S4) and 24 h (S24) are shown. Genes are listed by gene name, and those genes showing a similar expression pattern in the confirmatory experiments using the Unigene array are marked by a (C). Those genes that were represented on the Unigene array but did not exhibit the same expression pattern are marked as (NC). The data are expressed as the $\log_2$ of the expression ratio, and the key for intensity of expression is: -5 -2 1 2 >5 Grey bars signify missing values secondary to failure of a given gene spot to meet minimal intensity requirements for that experiment.

analysis were increased in *E. histolytica*-infected human colonic xenografts, and not *S. flexneri*-infected human colonic xenografts (Fig. 5). The only difference detected was in the expression of α-defensin, which was increased only at the 4 h time-point in the microarray analysis of *E. histolytica*-infected human colonic xenografts (Fig. 5A), but was elevated at both 4 and 24 h in the Real-time PCR analysis (Fig. 5A and B).

Discussion

The clinical and pathologic endpoints of severe *E. histolytica* or *S. flexneri* infection are clear – colitis, often with mucosal ulceration, resulting in dysentery. We used a transcriptional analysis to ask whether these endpoints reflect a stereotypic gut response to any invasive pathogen, or whether the intestinal response to *E. histolytica* and *S. flexneri* is pathogen-specific. Both *E. histolytica* and *S. flexneri* are human-specific pathogens, so we used SCID-HU-INT mice and infected human colonic xenografts to model each disease. Whereas a number of recent microarray analyses have focused on the interactions of pathogens with specific cell types *in vitro*, we were interested in the tissue response to infection *in vivo*, and used RNA isolated from sections of infected human colon. This gives a complex readout from all the constituent cells of the colon (e.g. epithelial cells, fibroblasts, smooth muscle cells, endothelial cells), as well as any influxing inflammatory cells, but offers the significant advantage of allowing us to see the results of cell–cell interactions, where the effects of cytokines or mediators produced by one cell type on the gene expression of another cell type

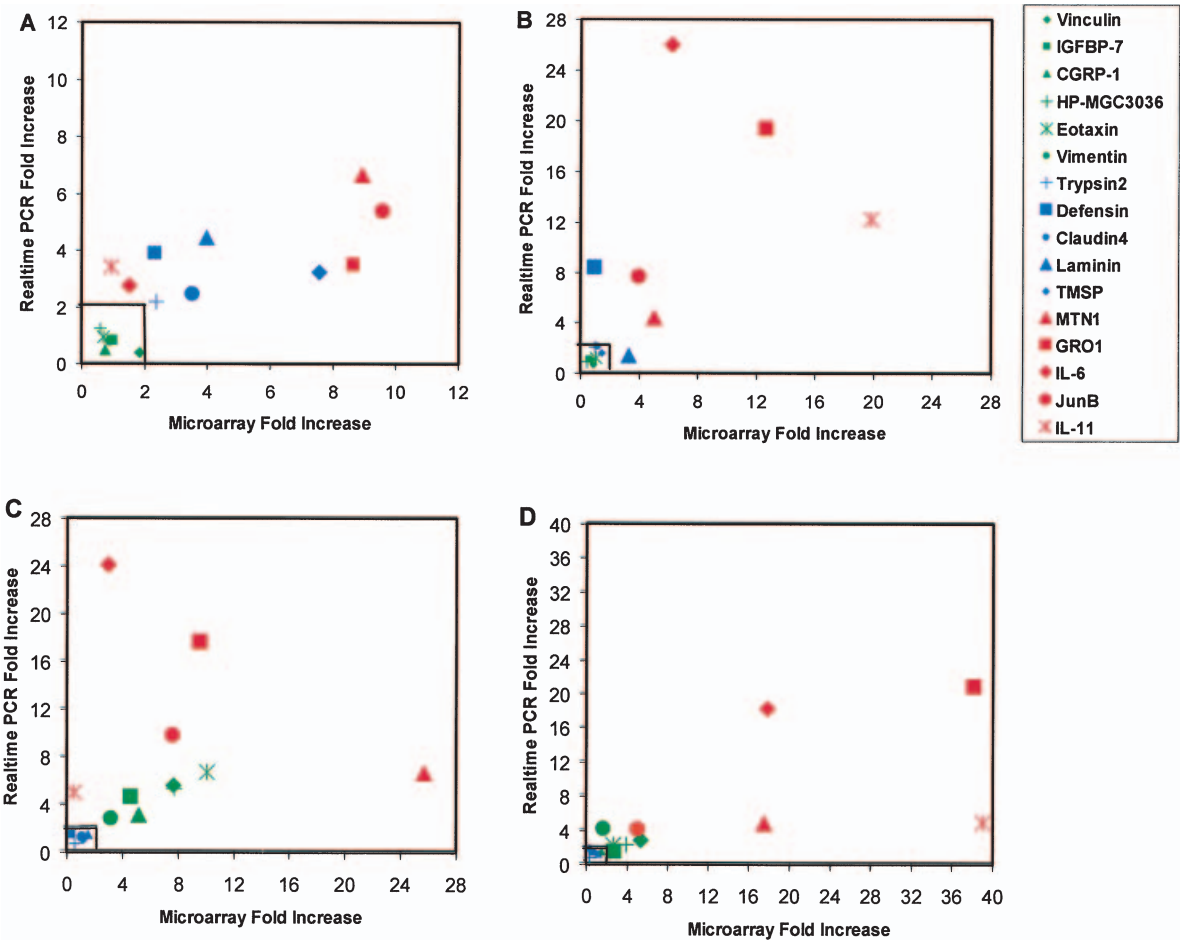

Fig. 5. Confirmation of differential gene expression by Real-time PCR analysis. The graphs show the correlation of the mean fold increase in message levels in *S. flexneri* or *E. histolytica*-infected human colonic xenografts compared to uninfected control human xenografts for a given gene by microarray analysis (*x*-axis) or by Real-time PCR (*y*-axis). Microarray values are the mean from the three experiments for each time-point, and Real-time PCR message values are the mean from three separate human intestinal xenografts for each time point. Genes selected for confirmatory Real-time PCR analysis that appeared to be increased in both *E. histolytica* and *S. flexneri* infection based on microarray results (indicated by red symbols) were metallothionein 1 L (MTN1), GRO1 oncogene (GRO1), interleukin-6 (IL-6), JunB (JunB), and interleukin-11 (IL-11). Genes selected for confirmatory Real-time PCR analysis that appeared to increased in *S. flexneri* infection alone (indicated by green symbols) were cysteine and glycine rich protein 1 (CGRP1), vinculin (vinculin), hypothetical protein MGC3036 (HP-MGC3036), insulin growth factor binding protein-7 (IGFP-7), vimentin (vimentin), and eotaxin (eotaxin). Genes selected for confirmatory Real-time PCR analysis that appeared to increased in *E. histolytica* infection alone (indicated by blue symbols) were alpha-3-defensin (defensin), trypsin 2 (trypsin2), claudin 4 (claudin4), laminin γ–2 chain (laminin), and transmembrane type 2 serine protease (TMSP). Results are shown for each gene in *E. histolytica* infection at 4 h (A), 24 h (B), and *S. flexneri* infection at 4 h (C) and 24 h (D). The boundary of twofold differential increase in expression in indicated for each graph.

will be detected. The chimeric nature of the SCID-HU-INT mouse introduces another layer of complexity into the analysis, as most of the colonic tissue, epithelial cells, fibroblasts, smooth muscle layers, are of human origin, whereas most of the inflammatory cells, and some of the endothelial cells will be of murine origin. We used human gene arrays for these experiments, as our primary focus was on the responses derived from colonic tissue, and we elected not to alter the stringency of our hybridization conditions to try and better detect altered expression of murine genes. However, we cannot exclude the possibility that some proportion of the human genes we detected that displayed differential expression are actually the homologous murine gene, expressed by mouse macrophages, monocytes, neutrophils or endothelial cells, resident in infected human colonic xenografts. Because our goal was to analyse the global intestinal response to infection, we do not consider the possible inclusion of such genes to be a disadvantage, but we recognize that it may introduce some bias into our results, as differentially expressed murine genes that have more sequence identity to the homologous human gene would be more likely to be detected in our analysis.

We found that there were pathogen-specific responses to *E. histolytica* and *S. flexneri* in human colonic xenografts, and these were most marked at the early time-

point, 4 h. By 24 h the transcriptional responses were much more similar, and were dominated by the increased expression of genes encoding molecules involved in inflammation and tissue repair. For each infection, some of the unique elements of the host response detected in the transcriptional analysis could be related to the known pathogenic mechanisms of *S. flexneri* or *E. histolytica*. For Shigella infection, the transcriptional analysis appeared to reflect the ability of intracellular *S. flexneri* to co-opt the cytoskeletal machinery of infected epithelial cells. *Shigella* entry into epithelial cells, and its subsequent cell to cell spread, has been studied extensively *in vitro*, and is dependent upon a number of host molecules, including actin, actin-related protein 2/3 complex (ARP 2/3) vinculin, myosin, and moesin (ezrin). (Tran *et al*., 2000) Strikingly, the expression of the genes encoding these proteins was specifically increased in *S. flexneri*-infected human colonic xenografts, consistent with bacterial utilization of these host pathways *in vivo*. The increased expression of other genes known to encode proteins that interact with actin, including annexin A1, gelsolin and thymosin B10 raise the possibility that these proteins could also play a role in Shigella infection *in vivo*. (Yu *et al*., 1993; Choe *et al*., 2002; Gerke and Moss, 2002) Shigella alteration of microtubule function has been reported, and we found alterations in dynein and tubulin gene expression in *S. flexneri*-infected human colonic xenografts. (Yoshida and Sasakawa, 2003) Vimentin, an abundant intermediate filament protein, is expressed and secreted by macrophages in response to bacterial infection, and we found increased expression of the vimentin gene in *S. flexneri*-infected human intestinal xenografts. (Mor-Vaknin *et al*., 2003)

We found an increase in the expression of the smooth muscle-specific genes encoding smoothelin, desmin, cysteine and glycine rich protein, and caldesmon 1, as well marked increases in genes encoding collagen (Col1A1, Col1A2, Col4A1, Col4A2, Col5A2 and Col6A3) in *S. flexneri*-infected human colonic xenografts. Because *S. flexneri* infection was generally confined to the mucosal region, without obvious involvement of the colonic muscle layers, the source of these gene products may be activated fibroblasts or the intestinal subepithelial myofibroblasts located in the lamina propria under epithelial cells. (Powell *et al*., 1999) The latter cells are known to play a role in the repair of epithelial damage and colonic ulcerations, and are activated by multiple mediators including COX-2, interferonγ, TNFα, IL-1β, insulin dependent growth factor 1 (IGF1), and TGFβ. Colonic biopsies from individuals with ulcerative colitis or Crohn's colitis also show increased expression of collagen genes, including Col1A1, Col1A2, Col4A2 and Col6A3, indicating that increased expression of these genes is a component of the tissue response to inflammation within the colonic mucosa. (Lawrance *et al*., 2001) Many of the genes activated in *S. flexneri* infection, including Col1A2, Col4A2, Col6A3, and the TGFβ-induced factor, are specifically induced in fibroblasts by TGFβ. (Verrecchia *et al*., 2001) The apparent lack of activation of some of these 'repair' genes in *E. histolytica*-infected human colonic xenografts may reflect a decreased activation of the TGFβ and the IGF pathways in amoebic infection, or simply reduced inflammation and mucosal damage in *E. histolytica* compared to *S. flexneri* infection.

The increased expression of a number of genes that could modulate the human colonic response to infection was detected in *S. flexneri*-infected human colonic xenografts. The regulators of G-protein signalling (RGS) negatively regulate G-protein coupled receptor signalling, but also can activate MAP kinase pathways in the absence of G-protein coupled receptor signalling via G pathways. Increased gene expression of two of the RGS proteins (RGS-1 and RGS-16) has been reported in tissues from animals septic from infection with Gram-negative bacteria (Panetta *et al*., 1999). We found increased expression of RGS1, RGS-16, and RGS-2 specifically in *S. flexneri*-infected human intestinal xenografts, suggesting a mechanism by which *S. flexneri* could influence the host responses by down regulating G-protein signalling or activating MAP kinase signalling pathways.

Shigella flexneri infected human xenografts showed increased expression of genes that are implicated in control of the cell cycle and programmed cell death. These included the transcription factor BTEP-1, the pro-apoptotic protein programmed cell death 5, and the antiproliferative BTG family member 2. Apoptosis can be seen in lamina propria cells obtained from rectal biopsies from individuals with acute shigellosis, and has been linked to bacterial LPS signalling via Toll receptor 2. (Aliprantis *et al*., 1999; Raqib *et al*., 2002) Whether the pro-apoptotic molecules identified in this transcriptional analysis, including programmed cell death 5 and galectin-1, play a role in this process remains to be determined.

There were fewer genes that showed increased expression specifically in *E. histolytica*-infected human colonic xenografts. This may reflect a less complex mode of pathogenesis for amoeba, related in part to their purely extracellular location. *Entamoeba histolytica* trophozoites added to a monolayer of mammalian cells exert a cytopathic effect, with detachment of cells from the monolayer. This effect, which may be key in allowing *E. histolytica* trophozoites to invade through the mucosal layer, is mediated by cysteine proteinases released from *E. histolytica* that digest extracellular matrix proteins (Keene *et al*., 1990; Zhang *et al*., 2000). Our data suggests that the cytopathic effect may be a requisite and unique element of amoebic pathogenesis *in vivo*. Adhesion of epithelial cells to the basement membrane requires integrin α6–β4 and laminin-5, which form tight molecular complexes linking the plasma

membrane of epithelial cells to the basement membrane (Falk-Marzillier *et al*., 1998). We found significantly increased expression of the genes encoding two of the components of laminin-5 (laminin β3 and laminin α2) and both integrin α6 and integrin β4 chains in *E. histolytica* infected human colonic xenografts, which, in the simplest interpretation, could be consistent with an attempt to maintain epithelial cell adhesion to the extracellular matrix by replacing proteins cleaved by *E. histolytica* cysteine proteinases. We also found increased expression of the genes encoding β-catenin, claudin 4, and junction plakoglobin; proteins that are comprise intercellular epithelial cell junctions. These results differ from those seen in inflammatory bowel disease, where a downregulation of the expression of junctional molecules with disease has been observed (Gassler *et al*., 2001). It is known that *E. histolytica* trophozoites can disrupt tight junctions between epithelial cells, suggesting that the increase in expression of some of the junctional proteins represents a compensatory response to specific effects of *E. histolytica* on epithelial cells. (Li *et al*., 1994; Leroy *et al*., 2000).

One mechanism for diarrhoea is altered ion transport by colonic epithelial cells. The trypsin serine proteases have been linked to increased Cl(–) secretion by colonic epithelial cells via their activation of the proteinase activated receptor 2 (PAR2) in human colon (Mall *et al*., 2002). We found increased expression of the genes encoding two trypsin serine proteases and PAR2 in *E. histolytica* infected human colonic xenografts. The serine proteases, prostasin (serine protease 8), and transmembrane serine protease 2 (TMPRSS2) were recently shown to directly regulate epithelial sodium channel (ENaC) expression (with prostasin activating Na^+ channels, whereas TMPRSS2 decreased sodium currents and ENaC protein levels). Expression of the genes for each of these proteases was increased in *E. histolytica*-infected human colonic xenografts (Donaldson *et al*., 2002). We also found increased expression of the gated chloride channel ClC-2, a molecule implicated in chloride secretion by intestinal epithelial cells (Mohammad-Panah *et al*., 2001). Whether any of these changes correspond to a physiologic role for these proteins and altered ion transport in *E. histolytica* induced diarrhoea remains to be determined.

Although we were able to detect genes that are specifically expressed in *S. flexneri* or *E. histolytica* infection, our results strongly suggest that most of the intestinal response is stereotypic, with the same patterns of gene expression seen in each infection. Not unexpectedly, given the histopathologic findings of inflammation and mucosal damage seen in both *E. histolytica* infection and *S. flexneri* infection, the stereotypic element of the response was dominated by genes encoding proteins mediating inflammation, stress responses, and tissue repair. Most of the major signalling and regulatory pathways that control or induce cytokine production were represented among the genes showing increased expression in infectious colitis caused by *S. flexneri* or *E. histolytica*. Induction of JNK and p38 MAP kinases was evident by the increased expression of genes in the AP-1 family (c-jun, c-fos, JunB, JunD), and p38 MKK3, respectively, whereas activation of the NF-κB pathway was demonstrated by the increased expression of the IκBα gene. Multiple genes known to be induced by TNFα or IL-1 (IL-1β, IL-6, IL-8, MCP-1, MCP-3, MIP-3-, Gro1–3, IκBα, ENA-78, and COX-2) were differentially expressed in *S. flexneri* and *E. histolytic*-induced colitis, consistent with an important role for these cytokines in the pathogenesis of dysentery and gut inflammation. (Seydel *et al*., 1997; Zhang *et al*., 2001;2003; Ciesielski *et al*., 2002; Kwon *et al*., 2002; Chen *et al*., 2003; Tsuzaki *et al*., 2003) Many of these genes also show increased expression in colonic biopsies from individuals with ulcerative colitis or Crohn's colitis (e.g. IL-1β, IL-8, IL-6, Gro1–3, lipocalin, MCP-1, interferon-inducible proteins, SOD-2, serum amyloid proteins) (Lawrance *et al*., 2001), again consistent with the concept that different initial injuries may induce gut inflammation through a limited number of pathways.

Genes linked to the adaptive response to hypoxia showed increased expression in both *E. histolytica* and *S. flexneri* infected human colonic xenografts, including the transcription factor HIF-1, and its target genes, lactate dehydrogenase A, enolase 1 and 2, fructose-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, HIF-1 responsive RTP801, and Glut-1 (solute carrier family 2, member 1). HIF-1 gene expression can be increased by TNFα under normoxic conditions, so it remains unknown at this point whether the activation of these genes reflects an important role for hypoxia/ischaemic injury in the pathogenesis of amoebic and bacterial dysentery (Mathan and Mathan, 1991), with direct triggering of HIF-1 through hypoxia, or whether activation of these genes is a component of the inflammatory response mediated by TNFα. (Scharte *et al*., 2003) Other genes known to be activated by stress/injury and also by cytokines, including the genes encoding superoxide dismutase, serum amyloid proteins, and the metallothioneins (MTs), were also increased in both *E. histolytica* and *S. flexneri* infected colonic xenografts. Metallothionein gene expression, which is regulated by multiple factors including metal-responsive transcription factors (MTF-1), IL-6 and TNFα (probably via AP-1), and γ-INF response elements, is known to be increased in sepsis, but has not previously been linked to infectious colitis (Sciavolino and Vilcek, 1995; Chinnaiyan *et al*., 2001). Interestingly, the expression of MT1H and MT1G was decreased in colonic biopsies from individuals with Crohn's colitis or ulcerative colitis compared to healthy controls (Lawrance *et al*.,

2001) suggesting MT gene expression may be a distinguishing marker for infectious colitis.

The colonic inflammation and the resultant tissue damage seen in *E. histolytica* and *S. flexneri* induced colitis arises in the face of multiple counter-regulatory measures. The gene encoding the SOCS-3 protein, which inhibits the actions of multiple cytokines, and is increased in the colonic mucosa of individuals with inflammatory bowel disease, as well as in immune-mediated and chemical-induced animal models of colitis, showed increased expression in both *S. flexneri* and *E. histolytica* infection (Greenhalgh and Hilton, 2002). Increased expression of the gene encoding the cytokine interleukin-11, which is known to inhibit IL-1, IL-6, and TNFα release from macrophages, and has recently shown efficacy in treating individuals with Crohn's disease, was seen at the late time-points in both *E. histolytica* and *S. flexneri*-infected human colonic xenografts (Sands *et al*., 2002). Activation of the MMP1, MMP12, and MMP17 genes was accompanied by increased expression of their inhibitors TIMP-1 and TFPI-2 (Herman *et al*., 2001). Even in the early phases of disease, before marked mucosal damage was present in some cases, genes encoding proteins involved in the induction and regulation of angiogenesis and fibrosis (e.g. cryptic gene, cysteine rich angiogenic inducer, connective tissue growth factor, angiopoeitin, stanniocalcin 1, and BMP antagonist) were already activated in both *S. flexneri* and *E. histolytica*-infected colonic xenografts (Bell *et al*., 2001). We know that the gut inflammation associated with either *E. histolytica* or *S. flexneri* infection eventually resolves, after antimicrobial therapy or host immune/inflammatory responses eliminate the causative organism. The endogenous inhibitors of cytokines and proteases, produced even in the initial phases of the inflammatory response, as well as the early induction and regulation of tissue repair and remodeling factors, may be critical in mediating this resolution.

In summary, our data show that from the host perspective, infectious colitis caused by either *S. flexneri* or *E. histolytica* results in the activation of highly conserved inflammatory, stress response, and tissue repair/regeneration pathways. By 24 h of infection, these responses dominate the transcriptional response in both *S. flexneri* and *E. histolytica*-infected human colonic xenografts. Many of these pathways are activated in inflammatory bowel disease and in intestinal ischaemia, so our molecular dissection of the colonic response to injury may shed light on a variety of intestinal diseases and provide new targets for controlling gut inflammation. On the background of these conserved responses, we were also able to detect transcriptional patterns that were specific for Shigella or *E. histolytica.* These specific pathways were most marked early in infection, consistent with the concept that the forms of injury may be unique, but once the epithelial cells are damaged, the gut responses become stereotypic. Some components of the unique patterns of gene expression in *S. flexneri* or *E. histolytica* colonic infection were consistent with pathogenic mechanisms that had been identified in *in vitro* studies, but had not previously been shown to occur *in vivo*. Other transcriptional findings represent previously unrecognized molecular responses to *E. histolytica* or *S. flexneri* infection, and may provide new insights into the pathophysiology of each disease. The results of this study led us to look at the effects of inhibiting TNFα in amoebic colitis (Zhang *et al*., 2003), and we anticipate that the products of other genes identified by this analysis will be tested for their role in the pathogenesis of *S. flexneri* or *E. histolytica* infection.

Experimental procedures

Bacteria and amoeba

Shigella flexneri WT2457T (provided by Dr F. Noriega, Center for Vaccine Development, University of Maryland School of Medicine) was grown on tryptic soy broth with aeration for 24 h before inoculation into SCID-HU-INT mice. *Entamoeba histolytica* strain HM1:IMSS was grown on BI-S-33 as previously described (Stanley *et al*., 1990).

SCID-HU-INT mice

Human colonic xenografts were placed into the subscapular region of 6–8 week old SCID mice as previously described (Seydel *et al*., 1997). Incisions were closed with Michel clips, and grafts were allowed to develop for 10 weeks before use.

Infection of human intestinal xenografts

Human colonic xenografts in SCID-HU-INT mice (groups of 4) were infected by direct intraluminal inoculation with either 1×10^6 *E. histolytica* trophozoites or 1×10^7 *S. flexneri*. A group of three donor-matched human colonic xenografts were sham infected by inoculation with media alone. At 4 or 24 h following infection, animals were sacrificed, and the grafts were removed for RNA isolation. The RNA samples from the four infected human xenografts were pooled, and the RNA samples obtained from the sham-infected human intestinal xenografts were pooled. A total of three separate experiments comparing four amoebic-infected and three donor-matched uninfected intestinal xenografts were performed for amoebic infection at 4 h, and three separate experiments comparing four amoebic infected and three donor matched uninfected human intestinal xenografts were performed for amoebic infection at 24 h. An identical protocol was used for *S. flexneri* infection, with three separate experiments comparing four shigella-infected human intestinal xenografts and three donor-matched uninfected human intestinal xenografts performed for shigella infection at the 4 and 24 h time-points. An additional confirmatory set of microarray experiments for each infection and each time point using four donor-matched human colonic xenografts for each group and arrays produced by Incyte was also performed.

RNA isolation

A 500 mg section of each human colonic xenograft was obtained and saved in 4 ml RNALater reagent (Ambion, Austin, TX). RNA was isolated using TRIZOL reagent (Invitrogen, Carlsbad, CA) and purified with RNeasy mini kit (Qiagen, Valencia, CA) following exactly to the manufacture's protocol. For the confirmatory Incyte array screening, poly A + mRNA was obtained from total RNA using the Oligotex spin column (Qiagen, Valencia, CA) exactly according to the manufacturer's instructions.

Probe synthesis

For the Stanford array analysis, probe synthesis was performed using the Genisphere 3DNA™ submicro labelling protocol as described by the manufacturer, using 5 μg of total RNA, and 3 μl of Cy3 or Cy5 RT primer (detailed protocol at: http://www.genisphere.com/pdf/submicro1x_8-15-02.pdf). For the confirmatory Incyte arrays, mRNA was first quantified using Ribogreen dye (Molecular Probes, Eugene, OR), then 25–100 ng of mRNA were separated on an Agilent 2100 Bioanalyzer to examine size distribution (Agilent Technologies, Palo Alto, CA). Subsequent steps of probe synthesis, hybridizations, scanning, and data acquisition were performed using the protocols described by Yue *et al*. (2001). Two hundred ng of purified RNA were converted to Cy3-(infected xenografts) or Cy-5 (uninfected xenografts) probe using a custom labelling kit (Incyte Genomics). Labelled Cy3 and Cy5 cDNA products were combined and purified on a size exclusion column, concentrated by ethanol precipitation, than resuspended in a hybridization buffer (Yue *et al*., 2001).

Array hybridization

Hybridization of labelled cDNA probes on the Stanford human array which contains approximately 43 000 spots was performed using the Genisphere protocol (vide supra). Spots on the Stanford array consist of 85.8% I.M.A.G.E. consortium clones (sequence verified human cDNA clones), 9.8% clones from the Cancer Genome Anatomy Project (CGAP), and 3.6% control spots (details at http://www.microarray.org/sfgf/jsp/home.jsp). Hybridization of the human Unigene 1 array (Incyte Genomics) which contains 9248 PCR products derived from unique clones from the human Unigene collection and proprietary clones from Incyte was performed using the protocol of Yue *et al*. (2001). After hybridization, microarrays were scanned with an Axon GenePix 4000 A fluorescent scanner (Axon Instruments, Foster City, CA) and Gene Pix image acquisition software (Axon) at 535 nm for Cy3 and 625 nm for Cy5. For Stanford arrays, the Scanalyze program (http://rana.lbl.gov/EisenSoftware.htm) was used to quantify signal and background intensity for each spot, and the ratio of normalized intensities was obtained as the differential expression ratio for the specific gene in the two RNA samples. Genes had to exhibit a greater than twofold difference than background intensity in at least one experimental condition to be considered for further analysis. The data was analysed using Microsoft Access™ software, and genes exhibiting a differential expression ratio ≥ 2 in two of the three experiments for *S. flexneri* infection at 4 or 24 h, and for *E. histolytica* infection at 4 or 24 h were considered positive. Data from the Access analysis was imported into the Cluster program (http://rana.lbl.gov/EisenSoftware.htm), log transformed, and ratios grouped using average linkage hierarchial clustering. Data are presented using Treeview software (http://rana.lbl.gov/EisenSoftware.htm) – genes are listed by gene name and each gene is only listed once, even if it had duplicates on the array, regardless of whether the accession numbers were identical. For Incyte arrays, the GemTools software (Incyte Genomics) was used to quantify signal and background intensity for each spot, and the ratio of the two corrected signal intensities was calculated and expressed as the differential expression ratio for the specific gene in the two mRNA samples (Yue *et al*., 2001). Global normalization was done by calculating a balance coefficient to correct variations in data, possibly the result of unequal amounts of sample, poor quality sample, or fluorescent dye fade. The average signal from all elements in the Cy3 channel was divided by the average signal from all elements in the Cy5 channel. The Cy5 signal for each element was then multiplied by the balance coefficient, before calculating the expression ratio (Cy3/Cy5). This gives rise to the balanced differential expression ratio. Data from Incyte indicates that for the same element under the same conditions, differential expression ratio values all fall between ±1.4 (Yue *et al*., 2001). For these experiments, we analysed the data using the cut-off recommended by Incyte (≥1.7) for differential expression.

Table 4. Primers utilized in Real-time PCR confirmatory assays.

Gene name	Forward primer	Reverse primer
Cysteine-glycine rich protein	CTCAGGGCAAAGGGAAAGGT	GGCCAGGGCTCTGTTCTTG
α-Defensin	CTCAGCAGCAGAATCCCAGAGT	AAAACATGGCCTGCTATTGCA
Hypothetical protein	TTCCAACCCCAAAAATATTCCA	CTTCGTCTTCTGAACGTGCCTAT
IGFBP-7	GTGCCATGCATCCAATTCC	CGGCACCTTCACCTTTTTTC
18S rRNA	AGTCCCTGCCCTTTGTACACA	GCCTCACTAAACCATCCAATCG
Trypsin2	ATCACCCTGGCAGGAATCCT	TGAGCCAGGCTGAGTGTGAA
Vinculin	GAGAAGCATGTTTGAAGACCAAGAA	AGATCACCAAAAAGAACCCAAAACT
Vimentin	AACAGCTTTCAAGTGCCTTTCTG	AAATCTTGTAGGAGTGTCGGTTGTTA
JunB	AAGTCCTTACCTCTTCCGGAGATG	GCCTGGCTCAACATGCTACTAA
Metallothionein- 1 l	CTGCAAAGGGACGTCAGACA	GCAAACGGGTCAGGGTTGTA
GRO1-α	ACATGCCAGCCACTGTGATAGA	CATTCCCCTGCCTTCACAAT
TMPRSS2	GGCGAAGAAGAGAAAGATGTGTTT	ACCCTTCCCCTGGTTGGA
Laminin-γ2	GGGATTCTGGCTGATGTGAAG	TCAAGAGCCTGGGTATTGTAGCA
Claudin4	AGTCCTCTGCCCCTTCCAA	CACAAAGAAGGAAGAGGAAAAACC
IL-6	CATGGGCACCTCAGATTGTTG	TGCCCAGTGGACAGGTTTC
Eotaxin	TGTCACGTGTGGGCAATGTT	CATCTTTGCCAGGACCTTTACAA
IL-11	GGGCTGCACCTGACACTTG	TGTTTCGCCCCCAGTACTG

Real-time PCR

Reverse transcription was performed using 2 μg total RNA, oligo(dT) as primers, and AMV reverse transcriptase (Fisher Research, Pittsburg, PA). Primers for PCR were designed using Primer Express Software (Applied Biosystems, Foster City, CA) and are shown in Table 4. Real-time PCR was carried out in a total volume of 10 μl using 200 nm of primer pairs, 5 μl of 2× SYBR Green PCR Mastermix (Applied Biosystems), varying concentrations of cDNA template, with conditions of the recommended universal thermal cycling parameters using a ABI 7700 (Applied Biosystems). Each reaction was run in triplicate. Relative quantification of gene expression was performed using the comparative CT method. The human 18S ribosomal RNA was used as an internal control and the uninfected xenograft as sample control.

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