C1

Lysozyme expression and synthesis in the avian gastrointestinal tract

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Lysozyme is a 14kDa cationic protein which is bacteriolytic and plays a role in innate immunity. In the gastrointestinal (GI) tract of the human and mouse, lysozyme is synthesised and secreted by specialised granular epithelial Paneth cells which are located in the crypts of the intestinal villi. The aim of this study was to investigate lysozyme expression and synthesis in the chicken (*Gallus gallus*) avian gastro-intestinal epithelium.

Lysozyme was identified in the gut mucosa of five day old birds using RP-HPLC, an antimicrobial assay involving a *Salmonella typhimurium phoP* mutant and MALDI-TOF mass spectrometry. To identify the intestinal cells synthesising the protein immunohistochemistry was performed on TS sections from the GI tract of 17 day old birds using a 1: 100 dilution of a polyclonal antibody to hen egg white lysozyme, and a secondary antibody conjugated to FITC. Comparable mouse GI sections were stained in parallel. In the mouse fluorescent staining was localised to the base of the intestinal crypts. In contrast staining was detected on the epithelial surfaces of the avian tissues and no staining was observed at the base of the intestinal villi. This suggested that in the avian gut lysozyme was synthesised by intestinal epithelial cells and not by specialised Paneth cells.

The GI expression of lysozyme was investigated using reverse transcriptase polymerase chain reaction (RT-PCR) and total RNA isolated from the GI tract of birds aged 4, 17 and 38 days. Three sets of primers were designed to amplify cDNAs encoded by the lysozyme C and chick lysozyme G (Gc) genes, and a third novel lysozyme G gene (Gk). cDNA products of the correct size were generated and confirmed by DNA sequencing.

These data show that the avian intestinal epithelial cells synthesise and secrete lysozyme per se and suggest that there are at least three different genes expressing lysozyme in the avian GI tract.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C2

Antimicrobial peptide expression and immunolocalisation within human palatine tonsils

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The antimicrobial peptides (AMPs) are short, highly positively-charged peptides of 20-40 amino acids in length with broad spectrum antimicrobial activity, believed to be important components of epithelial defence (Lehrer & Ganz 2002). The aim of the present study was to compare AMP expression and localisation between human palatine tonsils and HEp-2 cells from an epidermoid cancer of the larynx, an *in vitro* human epithelial model widely used to study tonsillitis (Eyal et al. 2003).

Palatine tonsils were harvested with ethical approval and after informed consent from 19 patients with recurrent acute tonsillitis (during disease-free phase) and 5 control subjects undergoing tonsillectomy for sleep disorders. The surface epithelium was immediately dissected and snap frozen in liquid nitrogen. Total RNA was isolated and AMP expression characterised using reverse transcription-polymerase chain reaction (RT-PCR) with primers specific for AMPs, and compared with results from the Hep-2 cell line. Fluorescent immunohistochemical techniques were used to localise AMPs within fresh frozen tonsil sections, imaged with a Leica TCS-NT laser scanning confocal microscope. Products of the predicted size were generated by RT-PCR for each AMP and identity (100%) was confirmed by nucleotide sequence. We confirmed human palatine tonsils as a site of human β-defensin 1, 2 and 3 and LEAP-1 expression (Chae et al. 2001; Harder et al. 2001). We also demonstrated expression of two additional antimicrobial peptides LL-37 (cathelicidin I) and LEAP-2. All six AMPs were expressed in all 26 tonsil samples. Human β-defensins 1-3 and LL-37 showed similar localisation by immunohistochemistry to the oropharyngeal surface and crypt epithelia. In contrast, the *in vitro* HEp-2 cell model expressed only human β-defensin 1 and 3. Induction of the four 'missing' AMPs in Hep-2 cells was not observed after a three hour challenge with 10^7 CFU/ml of M1 wild-type Group A Streptococcus. A range of AMPs are expressed in the human tonsillar epithelium, suggesting a role in protecting the tonsil epithelium from infections. Hep-2 cells do not express the same range of AMPs as tonsils, questioning use as a model in the study of tonsillitis.

Chae SW, et al. (2001). Acta Otolaryngol 121, 414-418.

Eyal O et al. (2003). FEMS Immunol Med Microbiol 38, 205-213.

Harder J et al. (2001). J Biol Chem 276, 5707-5713.

Lehrer RI & Ganz T (2002). Curr Opin Immunol 14, 96-102.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C3

P-glycoprotein protects epithelial cells from necrosis and apoptosis induced by the commensal bacterium *E.coli* C25.

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The efflux transporter, P-glycoprotein the mucosal surface of intestinal enterocytes, is able to limit the absorption of a broad range of luminal xenobiotics including many drugs. Little is known about the physiological significance or natural substrates of intestinal PGP, although the observation that PGP-knockout mice develop a high incidence of inflammatory bowel disease in the presence of normal colonic microflora (1) suggests that it

may protect the epithelial barrier from bacterial factors. To test this hypothesis, we investigated the effects of PGP activity on the response of epithelial cells to the gut commensal *E.coli* C25. Data are mean ± SEM. and significance tested by the Students t test. Exposure of the PGP-expressing colonic cell line, Caco-2 to 10^8 colony forming units/ml of *E.coli* C25 for 4 hours had no significant effect on cell viability compared to untreated controls, as judged by the % of cells staining with trypan blue (1.9 ± 0.4% vs 3.1 ± 0.7%; n=3). Co-incubation with C25 and the PGP inhibitor GF120918 (5μM) (2), caused a 3.4 ± 0.4 fold ($P \leq 0.05$, (n=3) increase in the level of necrosis compared to GF120918 controls. To further define the role of PGP in protecting epithelial cells from bacterial damage, the effects of C25 were compared in wild-type (MDCK-WT) and PGP-transfected (MDCK-PGP) kidney epithelial cells. C25 induced a significant increase in necrosis in MDCK-WT from 2.5 ± 0.5 to 11.2 ± 1.5 % ($P \leq 0.05$); in contrast there was no effect on MDCK-PGP cells 1.5 ± 0.4% to 2.4 ± 0.5% (n = 8). Addition of GF120918 (5μM) to MDCK-PGP cells restored their sensitivity to C25 (10.1 ± 1.1 fold increase in necrosis compared to GF120918 controls). C25 also increased apoptosis, measured by DNA fragmentation ELISA, in MDCK-WT by 7.2 ± 1.0 fold versus control but had little effect in MDCK-PGP (1.4 ± 0.3 fold; n = 3). In preliminary studies, diluted culture fluid from E coli C25 inhibited net transport of digoxin across epithelial monolayers by 42.7% suggesting that this strain secretes factors that are transported by intestinal PGP.

These data provide preliminary *in vitro* evidence that PGP is able to protect epithelial cells from the damaging effects of commensal bacteria. The mechanism by which PGP limits bacteria-induced apoptosis and cell death are currently unknown but may involve transport and exclusion of secreted bacterial factors. High levels of PGP expression in the lower gut may be important in maintaining homeostasis with the luminal microflora and may explain the development of inflammatory disease in this region following PGP deletion in mice.

Panwala CM *et.al.* (1998) J.Immunol. **161**, 5733-44.

Collett A *et.al.* (2004) Pharm Res. **21** 819-826.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C4

The extravacuolar life of an intravacuolar pathogen: salmonella can escape the vacuolar compartment of human epithelial cells

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Salmonella enterica sv. Typhimurium has developed a sophisticated way of communicating with its mammalian host to ensure its survival and multiplication throughout the process of infection. Although many Salmonella genes have been shown to be required to cause disease, little is known about host cell type specificity of Salmonella gene expression. In order to address this question we have used an integrative approach that combines DNA microarrays and a reporter system based on single copy gfp+ transcriptional fusions to determine the transcriptional profile of intracellular S. Typhimurium following infection of epithelial HeLa cells. Comparison of the Salmonella response to macrophage intracellular environment [Eriksson et al., 2003, Mol. Microbiol. 47: 103-118] showed that this pathogen has a distinct expression profile during infection of human epithelial cells. We have new evidence that the SPI1 and SPI2 pathogenicity islands are differentially expressed in macrophages and epithelial cells. Furthermore, we observed that Salmonella can escape the vacuolar compartment and multiply in the cytosol. This multiplication is accompanied by production of flagella within the epithelial cells. We will describe the major differences observed between the Salmonella response to macrophages and epithelial cells.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C5

Differential regulation of splice variants of the Zn transporter SLC30A5 in Caco-2 cells

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Two splice variants of the human *SLC30A5* Zn transporter gene include different first exons, indicating transcription from alternative promoters. Splice variant A is expressed at the apical membrane of transiently transfected Caco-2 cells (Cragg *et al.*, 2002), whereas the intracellular location of splice variant B is unknown. We previously reported that the 2.9 kb genomic region immediately upstream of the first exon of splice variant A failed to drive Zn-inducible expression of a reporter gene in the placental cell line JAR, consistent with our observations that SLC30A5 mRNA in JAR cells was not altered by changing the extracellular Zn concentration (Russi & Ford, 2003). We now present evidence that this genomic region drives Zn-regulated expression of a reporter gene in the human intestinal cell line Caco-2, consistent with earlier observations that increasing the extracellular Zn concentration increased SLC30A5 mRNA in Caco-2 cells (Cragg *et al.*, 2002). We also demonstrate that, whilst neither splice variant of *SLC30A5* is regulated by Zn in JAR cells, splice variant A is up-regulated and splice variant B is down-regulated in Caco-2 cells cultured at an increased Zn concentration.

A 2024 bp genomic region immediately upstream of the first exon of SLC30A5 splice variant A, including the 5' end of the cDNA, was subcloned into pBlue TOPO upstream of the *E. coli* β-galacotosidase reporter gene to give the plasmid pC2024. pC2024 was transfected into Caco-2 cells for transient expression. The Zn concentration of the culture medium was modified 24 hours post-transfection and β-galactosidase activity was measured in cell lysates after a further 24 hours. β-galactosidase activity expressed from pC2024 in Caco-2 cells at 100 μM Zn was greater than that expressed at 3 μM Zn (5.2 ± 0.8 compared with 2.4 ± 0.5, mean ± SEM in units of fluorescence/mg protein; n=12, $P < 0.05$ by Student's *t* test). Analysis of RNA by north-

ern blotting followed by denistometric analysis of band intensities revealed that in Caco-2 cells *SLC30A5* splice variant A was up-regulated 2.0-fold but splice variant B was down-regulated 2.2-fold at 100 μM compared with 3 μM Zn. Western blotting revealed a parallel differential regulatory effect of two different molecular weight species at the protein level. In JAR cells neither splice variant was regulated by the change in Zn concentration. The expression of differentially regulated *SLC30A5* splice variants, possibly mediated through the use of alternative promoters, may contribute to cellular and/or systemic Zn homeostasis.

Cragg RA *et al.* (2002) *J Biol Chem* **277**, 22789-22797

Russi RM & Ford D (2003) *J Physiol* **547P**, C52

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C6

Studies on the proton coupling mechanism of the rabbit epithelial H+/peptide transporter PepT1, expressed in *Xenopus* oocytes.

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The epithelial electrogenic H+/peptide symporter PepT1 mediates the uptake of di-, tri-peptides and peptidomimetics in the intestine and kidney, reviewed by Meredith & Boyd (2000). A recent study (Meredith 2004) has shown that substitution of the conserved argine in position 282 (TM7) of PepT1 with a glutamate (R282E-PepT1) results in a transporter that is capable of transporting the dipeptide [^{3}H]-D-Phe-L-Gln but unlike the wild type the uptake is independent of extracellular pH. Therefore Arg-282 is considered to be involved in the proton gating of the transporter, possibly by charge pairing to another negatively charged residue.

Based on these findings we investigated which residue interacts with Arg-282. A conserved aspartic acid (D341, TM8) was considered a putative charge pair candidate due to its structural proximity, and its negative charge.

A single D341R-PepT1 and a double R282E/D341R-PepT1 mutant were generated in rabbit PepT1 using site-directed mutagenesis (Quickchange, Stratagene). The two mutations were confirmed by DNA sequencing. cRNA was microinjected and the uptake of the hydrolysis-resistant dipeptide [^{3}H]-D-Phe-L-Gln was measured as previously described (Meredith 2004). The controls employed were non-injected, wild type (WT)-PepT1 and R282E-PepT1 expressing oocytes. All data are normalized (pH 5.5 = 1) and expressed as mean ± S.E.M., n = 4-7 experiments of minimum 5 oocytes per experiment (Figure 1). The comparison between uptakes in different pH values was made using paired *t*-tests.

Uptake of the dipeptide D-Phe-L-Gln into oocytes expressing the single D341R mutant was found to be affected by extracellular acidification in a manner similar to the WT-PepT1 (alkalization from pH 5.5 to pH 7.4 results in a 3-fold statistically significant reduction of D341R-PepT1 uptake, $P < 0.005$). The R282E-PepT1 uptake remained constant whilst the pH was increased, in agreement with the published data. Interestingly, the double mutant R282E/D341R-PepT1 appears to follow the pH dependency of the WT transporter (3-fold statistically significant reduction of uptake in pH 7.4 compared to uptake in pH 5.5, $P < 0.005$).

Taken together, these results suggest that the pH independence of uptake by R282E-PepT1 is restored to a wild type like behaviour by the double mutation R282E/D341R-PepT1. They further suggest that introduction of the second mutation compensates for the R282E mutation by re-establishing a charge pair.

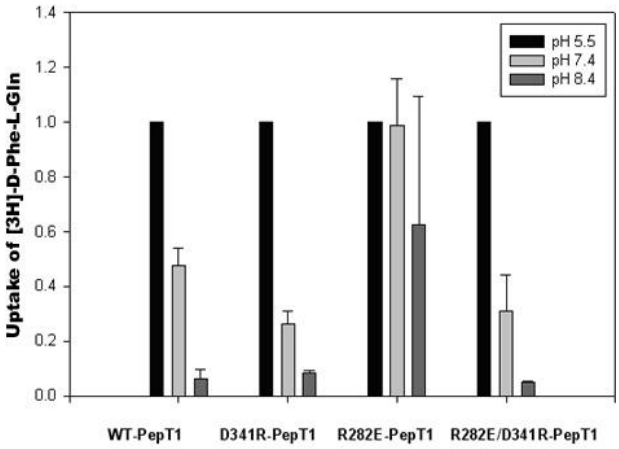

Figure 1: Normalized data plotted to describe the effect of extracellular acidification in the uptake of the dipeptide [3H]-D-Phe-L-Gln by WT-PepT1, by D341R-PepT1, by R282E-PepT1 and by R282E/D341R-PepT1 mutants.

Meredith D & Boyd C.A.R. (2000) *Cell Molecular Life Sci* ***57****, 754-758.*

Meredith D (2004) J. Biol. Chem. **279**, 15795-8.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C7

Identification and localisation of a UT-B facilitative urea transporter in bovine rumen

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The process of urea nitrogen salvaging (UNS) is vital for maintaining nitrogen balance in ruminant animals, such as cattle and sheep (Lapierre & Lobley 2001). An important step in UNS is the passage of urea across the rumen epithelia. Although the pathway is not yet clearly understood, the involvement of facilitative urea transporters has previously been indicated (Marini & Van Amburgh 2003). In this study, we characterize a bovine UT-B urea transporter ortholog and investigate the role of UT-B in the bovine rumen.

We obtained a 2234bp bovine UT-B cDNA clone, BE665260, from BACPAC Resources (CHORI, USA). The identified open reading frame (75-1229bp) encoded a 384 amino acid protein, bUT-B, which shared 79% homology with human UT-B (GenBank accession number Q13336). When expressed in *Xenopus* oocytes, bUT-B produced a 4-fold increase in urea transport ($P<0.001$, ANOVA) that was significantly inhibited by 500mM phloretin ($P<0.01$, ANOVA) or 2mM thionicotinamide ($P<0.01$, ANOVA). Rumen tissue was obtained from humanely killed cattle (*Bos taurus*). Northern analysis detected a 3.8 kb UT-B transcript in bovine rumen, the presence of which was further confirmed by RT-PCR analysis. Immunoblotting studies of bovine rumen tissue, using a mouse UT-B antibody (Stewart *et al.* 2004), detected a 45-50kDa UT-B protein. Using 10μm sections of methanol fixed rumen epithelium, UT-B was immunolocalised to the plasma membrane of cells of the stratum basale, stratum spinosum and stratum granulosum; the stratum corneum was negative. Functional studies of trans-epithelial transport across rumen epithelium showed that bi-directional fluxes of 14C-labelled urea were similar (Jlumen to blood 19.5 ± 2.5 nmol.cm-2.hr-1 (SEM), n=15 measurements from 4 animals, Jblood to lumen 18.7 ± 2.5 nmol.cm-2.hr-1(SEM), n=15) and exceeded simultaneously measured 3H-mannitol fluxes by 2.5 fold and 1.97 fold respectively. Phloretin (1mM) inhibited a component of the trans-epithelial urea flux, suggesting that additional pathways to UT-B (both mediated and passive) are likely to exist.
We therefore conclude that UT-B is present in the bovine rumen and is likely to participate in trans-epithelial urea transport. As such, bovine UT-B may play a role in the UNS process.

Lapierre H & Lobley GE (2001) *Journal of Dairy Science* **84,** E223-E236.

Marini JC & Van Amburgh ME (2003) *Journal of Animal Science* **81,** 545-552

Stewart GS *et al.* (2004) *Gastroenterology* **126, 765-773**

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C9

Role of VT (verotoxin) in interaction of enterohaemorrhagic Escherichia coli (EHEC) with intestinal epithelium.

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Enterohaemorrhagic Escherichia coli (EHEC), particularly of serotype O157:H7, are the most common cause of haemorrhagic colitis (HC) which can lead to life-threatening haemolytic uraemic syndrome (HUS) in humans. Verotoxins (VT; or shiga-like toxins) are major virulence factors and are key determinants in the patho-physiology of EHEC infections in humans. Cattle are an important reservoir host for EHEC in which infection occurs asymptomatically. Consequently the significance of virulence determinants, including VT, in the bovine host is obscure. Initial work identified potential contribution of VTs to bacterium-host interactions and this investigation aimed to characterise roles of VT in colonization of bovine intestinal epithelium by EHEC. For this, adherence of a panel of wild-type and mutant (VT- negative) EHEC strains to primary epithelial cells from the most terminal part of rectum (the principal site of EHEC colonization in cattle) was conducted (tissue obtained from the abattoir). Carriage of VT was associated with greater adherence to epithelium as demonstrated by higher capacity to form micro-colonies. Pre-treatment of cells with VT produced a similar phenotype. VT exhibited further effects on epithelium through reduction of secretion of IL-8, an important epithelial inflammatory mediator. VT of EHEC therefore do not show classic cytotoxicity for bovine intestinal epithelium but do exert pleiotropic effects on these cells, by modifying epithelial physiology hence enabling EHEC colonisation.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C10

A decrease in intracellular ATP mediates the inhibitory effect of hypoxia on the epithelial sodium channel (ENaC) expressed in *Xenopus laevis* oocytes.

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Hypoxia is thought to downregulate ENaC in alveolar epithelia and has been implicated in the formation of and failure to clear pulmonary oedema at high altitude. However the mechanisms involved remain unclear. Channel trafficking, direct channel modification and altered subunit transcription have all been implicated, but the evidence is conflicting. Recently, it has been reported that in 3T3 cells stably transfected with ENaC, channel activity ran down when the cells were dialysed with an ATP-free solution (Ishikawa *et al.* 2003). Here we examined the effect of hypoxia on rat ENaC heterologously expressed in *Xenopus laevis* oocytes, and sought to see whether this was related to a change in ATP levels using the two-electrode voltage clamp technique, a surface expression and a luciferase-based ATP assay. Data are given as means ± S.E.M.; significance was evaluated using the appropriate Student's *t* tests.
Following 18 hours of continuous exposure to anoxia, amiloride-sensitive currents (ΔI_{Ami}) in ENaC-expressing oocytes averaged 0.2 ± 0.0 μA (n = 83) compared with 5.7 ± 0.7 μA in time-matched normoxic oocytes (n = 74; $p < 0.001$). The effect of anoxia was reversible and after 6 hours of re-exposure to normoxia, ΔI_{Ami} averaged 5 ± 0.5 μA (n = 64). Despite a reduction in ΔI_{Ami} by 85.1 ± 2.0 % ($p < 0.001$) in anoxic (n = 20) compared to normoxic oocytes (n = 19), channel surface expression did not differ between the two groups (n = 37 and 35, respectively). Measurements of cytosolic ATP in normoxic oocytes averaged 2.4 ± 0.5 mM, compared with 0.9 ± 0.2 mM in anoxic oocytes

(n = 6 each; $p < 0.05$). Injection of exogenous ATP in anoxic oocytes resulted in a partial recovery of ΔI_{Ami} within three hours; in these oocytes ΔI_{Ami} averaged 51.7 ± 4.4 % of that seen in anoxic oocytes re-exposed to normoxia.

Using a Na^+-free perfusate to prevent rundown of channel activity as described by Volk *et al.* (2004), continuous current recordings were made over a 30-minute period. When the metabolic inhibitor sodium azide was added to the perfusate, we observed a 77.6 ± 4.7 % rundown in ΔI_{Ami} (n = 21; $p < 0.001$). There was no change in ENaC surface expression following incubation with sodium azide, thus mirroring the effects seen with anoxia.

Together these data suggest that $[ATP]_i$ is essential to maintain ENaC activity and that the reduction in $[ATP]_i$ during anoxia is responsible for the decrease in ENaC activity.

Ishikawa T *et al.* (2003) *J Biol Chem* **278**, 38276-38286

Volk T *et al.* (2004) *Pflügers Arch* **447**, 884-894

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C11

A role for a fimbriae-associated protein in Streptococcal adherence to human tonsil tissue

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Group A Streptococcus (GAS) is the most common pathogen identified in cases of recurrent acute tonsillitis. Short (~100 nm) cell-surface fibrils that interact with host tissues have been well characterised in GAS, but recently we observed that GAS can also express polymeric fimbriae extending for >1 μm from the surface (WD Smith, RJ Smith and MA Kehoe, unpublished). Similar surface appendages have been associated with adhesion in Gram-negative organisms such as *Salmonella typhimurium* and *Escherichia coli*. In this study, we have investigated the role that a fimbriae-associated protein (FimAP1) may have in GAS adherence to tonsil epithelium.

Human tonsil tissue was obtained, with informed consent and ethical approval, from patients undergoing tonsillectomy for recurrent acute tonsillitis (N=4). All specimens were transported to the laboratory within 1h of excision. From each tonsil, 12 primary explants of surface epithelium were incubated in RPMI-1640 media, supplemented with 10% foetal calf serum and 1% non-essential amino acids, at 37ºC with 5% CO_2. Wild-type M1 GAS (n=6) or ΔFimAP1-GAS (n=6), both at a concentration of 10^7 colony forming units (CFU)/ml and expressing green fluorescent protein (GFP) to allow visualisation, were added to the explants for 4h. Following incubation tonsil tissues were fixed, blocked with 0.5% bovine serum albumin and stained for cytokeratin 14, a tonsil epithelial cell marker (Clark et al. 2000), revealed by red-fluorescent Alexa Fluor 568-labelled secondary antibodies. Human epithelial HEp-2 cells were incubated with the same strains of Streptococcus. Explants were examined by laser scanning confocal microscopy.

Examination of growth media from all explant models confirmed GFP fluorescence of both wild type and ΔFimAP1-GAS. On examination of tissue samples it was observed that all tonsil explants from all 4 patients showed adherence of wild type GAS to the surface epithelium after 4h. However, the ΔFimAP1-GAS failed to bind to any tonsil samples. Contrasting results were observed in HEp-2 cells. Both wild-type and ΔFimAP1-GAS adherence to HEp-2 cells was observed.

Bacterial adherence and colonization is a common requirement prior to infection. The interaction of GAS, a common cause for recurrent acute tonsillitis, with tonsil surface epithelium is believed to occur via a number of adhesins (Jenkinson & Lamont 1997). The inability of ΔFimAP1-GAS to bind to tonsil tissue suggests an essential role for FimAP1 in Streptococcal/tonsil interaction.

Clark MA et al. (2000) Histochem Cell Biol 114, 311-321

Jenkinson HF & Lamont RJ (1997) Crit Rev Oral Biol Med 8, 175-200

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC1

Oxygen-dependent membrane transport in articular chondrocytes

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Articular cartilage is avascular and relatively hypoxic, but is habitually studied at ambient O_2 levels. Reduction in O_2 tension, however, inhibits ATP production in articular chondrocytes (Lee & Urban, 1997), and O_2 tension per se profoundly modulates membrane transport in other tissues (Gibson *et al.*,, 2000). Both may alter ion homeostasis, and hence cell function. pH and Na^+ are particularly important. Intracellular pH (pH_i) affects matrix turnover, whilst the Na^+ gradient, maintained by the Na^+/K^+ pump, is used for many secondary active processes including the major pH regulatory process, Na^+/H^+ exchange (NHE). Here we investigate the effects of O_2 tension on chondrocyte Na^+ and H^+ homeostasis.

Cartilage slices were taken from bovine and equine fetlock joints of animals humanely killed for other purposes (under Home Office guidelines). Chondrocytes, isolated by collagenase digestion, were incubated at 21% or 0% O_2 for 10 min before assaying ion transport at 37°C. pH_i was determined fluorimetrically with BCECF (Wilkins & Hall, 1992), using NH_4Cl to alter pH_i. Ouabain-sensitive ^{86}Rb influx was used as a measure of Na+/K+ pump activity. Salines were buffered with HEPES (10 mM), in nominal absence of CO_2 / HCO_3^-.

Steady state pH_i was 6.93±0.15 and 6.89±0.07 in normoxia and anoxia, respectively; buffering capacity, assessed by NH_3 rebound, was 38.61±4.10 mM.(pH unit)$^{-1}$ in O_2 vs 28.79±8.97 in N_2; amiloride (100μM)-sensitive H^+ efflux during recovery from acid load was 1.47±0.16 mol.(l cells.h)$^{-1}$ in O_2 and 1.12±0.25 in N_2 (all means±S.E.M., n>4). NHE activities were also confirmed using $^{22}Na^+$ influx (data not shown). None of the above changes was statistically significant (tested using Student's t-test). Na^+/K^+ pump activity, however, was 31.8±5.7 and 59.5±9.9 nmol.(10^6 cells.h)$^{-1}$ in oxygenated and deoxygenated cells, respectively (n=3; $p<0.05$).

ATP production by chondrocytes is largely glycolytic. It is reduced in anoxia, a "negative" Pasteur effect, correlating with inhibition of metabolic events such as matrix synthesis, although steady state ATP levels are largely maintained (Lee & Urban, 1997). We show that H^+ regulation and NHE activity were little affected by short term anoxia but Na^+/K^+ pump activity was substantially elevated. Thus reduction in ATP consumption is not uniform across all cell functions. We speculate that, unlike matrix synthesis, H^+ and Na^+ homeostasis are critical for cell survival, accounting for their relative protection during anoxia.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC2

Effect of peroxynitrite on K^+ transport in human red blood cells

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A number of oxidants increase the K^+ permeability of red blood cells (RBCs, Olivieri *et al.*, 1993). One of these is peroxynitrite. This powerful oxidant, produced in vivo from nitric oxide and superoxide, increases both Cl^- dependent and Cl^- independent K^+ flux (Grzelak *et al.*, 2001). It may be involved in RBC dehydration, particularly important in sickle cell disease. Little is known, however, about how it interacts with other important regulators of K^+ permeability.

RBCs, isolated from heparinised blood samples (from consenting volunteers with ethical permission) were reacted with peroxynitrite at pH 7.15, 37°C, c.4% haematocrit for 10 min in isotonic saline (composition in mM: NaCl 100, Na_2HPO_4 / Na_2HPO_4 50 mM). K^+ influx was then measured +/- Cl^- (replaced with NO_3^-) using $^{86}Rb^+$ was used as a K^+ congener, and ouabain (100 μM) and bumetanide (1μM), to obviate influx via Na^+/K^+ pump and Na^+-K^+-$2Cl^-$ cotransporter. Cl^- dependent K^+ influx was taken as a measure of K^+-Cl^- cotransporter (KCC) activity.

K^+ influx in control cells, or cells treated with decomposed peroxynitrite, was <0.15 mmol.(l cells.h)$^{-1}$ +/- Cl-, increasing to c.4.00 and c.3.00, respectively, with a KCC activity c.1, following 1 mM peroxynitrite. Thus, as reported, peroxynitrite increased K^+ transport by both KCC and Cl^--independent K^+ pathways. After treatment with peroxynitrite, the following results were obtained: (1) KCC activity fell when glucose levels were increased, with inhibitions of 54±4% and 94±15% at 4 and 5 mM glucose, respectively (means±S.E.M., n=3 or 4); (2) when incubated in N_2 (instead of air), influxes ± Cl^- decreased by c.70% and KCC activity fell from 1.19±0.35 to zero; (3) KCC activity was zero, 1.10±0.38 and 1.66±0.26 at pH 7.4, 7.2 and 7.0, respectively; (4) KCC was unaffected by reduction in osmolality from 300 to 257 mOsm.kg^{-1}; and (5) pretreatment with calyculin A inhibited KCC fully, post treatment inhibited it by about 40±24%.

Peroxynitrite-stimulated K^+ transport was therefore inhibited by glucose (and also other -OH containing compounds including sucrose and MOPS, data not shown) and O_2 removal, whilst KCC activity was unaffected by cell volume but remained pH-sensitive. Functional protein phosphatase activity was required for stimulation. These findings emphasise, in particular, the interaction of peroxynitrite with protein kinase / phosphatase enzymes regulating aspects of membrane permeability, especially KCC activity.

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PC3

Nitric oxide and transforming growth factor-beta are not responsible for LPS-induced proliferation in fetal rat lung explants

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Infection or inflammation resulting in preterm delivery can promote lung development. Many components of the inflammatory response could potentially be involved in this beneficial effect. Bacterial endotoxin can induce experimental inflammation in fetal lung explants and we have previously shown that lipopolysaccharide (LPS) increased airway branching, partly due to release of nitric oxide (NO) (Rae and Land, 2004). Here we show that the response to LPS was concentration-dependent, with high and low concentrations having opposite effects. Untreated lung explants from gestation day 14 rats (killed humanely) increased in size over 96 hours in culture and were characterised by the formation of cyst-like structures (airway surface complexity 0-96 hours, ASC = 1.54±0.11, n = 9, all values are Mean±S.E.M.). Exposure to 2 μg/ml LPS induced an increase in airway branching (ASC = 2.01±0.14, n=9, $P<0.05$, ANOVA, post hoc Tukey's HSD), whereas 10 μg/ml LPS caused proliferation throughout the lung, and decreased airway branching (ASC = 1.01±0.17, n=16, $P<0.01$ from control and 2 μg/ml LPS). Proliferation after exposure to 10 μg/ml LPS was not caused by increased NO release, which was lower than after exposure to 2 μg/ml LPS (control 10.13±1.05 mM Nitrite per mg protein; 2 μg/ml LPS 72.02±9.47; 10 μg/ml LPS 35.67±5.31, $P<0.01$ for 0-2 μg/ml LPS and 2-10 μg/ml LPS, n=20) and was not affected by iNOS inhibition ($P>0.05$). Exogenous NO released by diethylenetriamine/NO adduct (DETA-NO, 0.5 mM) resulted in 40-80 fold higher (n=10) NO concentration than LPS, but had no branching or proliferative effect on lung explants, indicating that NO alone was not responsible for this effect.

LPS caused activation of the transforming growth factor-beta (TGF-β) pathway, as demonstrated by western blotting, showing nuclear translocation of the SMAD4 signalling molecule. TGF-β is a negative regulator of airway branching and prevents LPS-evoked NO release via inhibition of iNOS (2 μg/ml LPS + 100 ng/ml TGF- β1 - 20.27±4.4 μM Nitrite per μg protein, n=4, $P<0.05$ from 2 μg/ml LPS). Although TGF-β inhibits epithelial proliferation, it can also cause thickening of pulmonary mesenchyme (Zeng et al., 2001). However, the LPS-induced proliferation was not prevented by co-incubation with TGF-β neutralising antibodies. This suggests that neither NO nor TGF-β alone were responsible for the proliferative effects seen when fetal lung explants are exposed to 10 μg/ml LPS, and we expect that this is caused by the release of other growth factors after exposure to LPS.

All procedures accord with current UK legislation

Rae C and Land SC (2004). J. Physiol 555P, PC114

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This work was supported by the Medical Research Council, Tenovus (Scotland) and the Anonymous Trust

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC4

Functional Organisation of the Bovine Rumen epithelium

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The bovine rumen epithelium is a major site for absorption of rumen microbial fermentation products (e.g. volatile fatty acids) (Stevens and Settker, 1966, Steven and Marshall, 1970) but may also contribute to maintenance of an optimal milieu for rumen micro-organisms. Here we define a transport model for this epithelium by using electron and light microscopy combined with immunocytochemistry.

Bovine rumen samples were obtained from a commercial abattoir. After SDS-PAGE separation of proteins from bovine rumen epithelial homogenates, Western-blotting demonstrated the expression of claudin 1 (an integral plasma membrane tight-junctional protein), connexin-43, (gap-junction monomer), and the catalytic alpha subunit of the Na+-K+ ATPase.

After methanol-fixation and cryo-sectioning, four cell layers could be distinguished in the epithelium, namely the stratum corneum, the stratum ganulosum, the stratum spinosum and the stratum basale. Claudin 1-immunostaining showed a peripheral cellular staining consistent with plasma membrane and was most intense at the cells of the stratum granulosum. Staining intensity decreased through the stratum spinosum to the stratum basale which showed only weak staining. The stratum basale was negative. Morphological identification of occluding tight junctions at the outermost layer of the stratum granulosum was confirmed by electron microscopy

Connexin-43-immunostaining was most intense at the stratum granulosum and stratum spinosum decreasing towards the stratum basale, staining was consistent with plasma-membrane staining.

There was intense immunocytochemical staining of the stratum basale for the Na+-K+ ATPase, with weak staining of the stratum spinsosum. Both the stratum granulosum and the stratum corneum were devoid of apparent staining. Foci of intense staining of the mitochondria-rich stratum basale cells in the region of the basement membrane were associated with extensive infoldings and interdigitations of the basement membrane and lateral space between stratum basale cells respectively. Thus polarity of Na+-K+ pump distrubution within the stratum basale cell reflects membrane amplification.

We conclude that epithelial barrier function may be attributed to the stratum granulosum, whilst cell-cell gap junctions allow diffusion to interconnect the barrier cell layer with the stratum basale where Na-K pump density is concentrated.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC5

Host epithelium and pathogen lipopolysaccharide interactions; lessons from lung transplantation for Cystic Fibrosis

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Background *Burkholderia cepacia* complex (BCC) organisms are important pathogens in Cystic Fibrosis (CF). They infect the CF lung and paranasal sinuses and are resistant to most antibiotics. Lung transplantation is a recognised treatment for CF patients with end stage disease and BCC infections. Lipopolysaccharide (LPS) is an important virulence determinant of Gram negative organisms and the CF epithelium appears to induce host-specific LPS changes in infecting pathogens. We have recently demonstrated CF specific lipid A conformational changes in BCC LPS which are likely to increase resistance to host cationic proteins. Early after lung transplantation pre-transplant organisms are often isolated in the lung lavages of transplanted CF patients probably reflecting infection of the donor lungs from the recipients upper airway. We hypothesized that persisting strains of BCC found in post lung transplant lung lavages may demonstrate conformational change in LPS reflecting the change from a CF to non-CF milieu.
Methods We tested paired BCC strains collected from U.K. CF patients transplanted at the Freeman Hospital who had pre-transplant BCC infection and who also had BCC infections present in the post-transplanted lung recovered at routine post operative lavages one week after transplantation. Using PCR techniques we were able to demonstrate the persistence of the pre-transplant strain. This was done initially by demonstrating the same BCC genomovar (sub-species) grouping pre and post transplant using genomovar specific primers and secondly by pulsed field gel electrophoresis using macrorestriction fingerprinting. The lipopolysaccharide from paired isolates were then compared by preparing SDS-gels that were silver stained for LPS.
Results We were able to demonstrate the persistence of pre-transplant BCC infecting organisms in 4 patients transplanted at our centre using the above PCR techniques. In these patients there were changes in migration patterns of the LPS when comparing pre and post transplant strains.
Conclusions Whilst prior data from our lab and others suggest lipid A and LPS changes are present in organisms isolated from CF airways it has been unclear if these changes remain fixed or are plastic to a changing environment. Our data suggests that this is a dynamic process with LPS changes within one week of transplantation which could be therapeutically useful. Further work assessing changes in the proinflammatory activity of LPS and lipid A conformation extracted from the paired pre and post transplant strains is under way.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC6

Transcriptional Profiling of Tracheal Epithelial Cells in Response to Infection with *Pseudomonas aeruginosa.*

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Cystic Fibrosis (CF), the most common lethal genetic disease of Caucasians, is caused by mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR). In relation to the lungs, this disease is characterised by mucus build-up, airway inflammation and microbial infection. *Pseudomonas aeruginosa* is the most dominant bacterial pathogen causing chronic lung infections in CF patients and is a significant contributor to morbidity and mortality in these individuals. *P. aeruginosa* is a versatile opportunistic pathogen producing an arsenal of virulence factors that enhance survival within the host by sequestering nutrients, contributing to tissue damage and evading the host immune response.
In this study, we report data from transcriptome profiling of epithelial cells in response to infection with wild-type and mutant strains of *P. aeruginosa*. The CFTE29o- cell-line, derived from tracheal epithelium from a CF patient is homozygous for the most common CFTR mutation, delta F508. *P. aeruginosa* was co-cultured with these cells at a multiplicity of infection of 50:1. Total RNA was extracted from CFTE29o- cells 1.5h post-infection and prepared for Affymetrix GeneChip® analysis as per manufacturer's instructions. Changes in gene expression in cells co-cultured with wild-type or mutant bacteria relative to no bacteria were analysed using GeneSight® software (Biodiscovery). mRNA transcript levels that changed by at least 2-fold in response to infection were considered significant.
In response to infection with wild-type *P. aeruginosa*, approximately 3% of the 22,215 represented genes showed a significantly altered expression 1.5h post-infection. Initial analysis indicates that the expression of genes involved in transcriptional regulation (ATF3, IFR5, MADH6, COPEB and KLF2), cytoskeletal organisation (ARHB, CDC42) and inflammatory response (interleukins, chemokines and TNF superfamily members) are altered in response to *P. aeruginosa*. To further investigate the differential regulation of a number of transcription factors at this early infection timepoint, temporal expression of these and additional genes in response to infection is being assessed. These changes in the expression profile of CFTE29o- cells undergoing infection indicate novel response mechanisms upon infection with *P. aeruginosa*. Investigation of the host response to *P. aeruginosa* strains, mutated in key virulence-related genes, has also been carried out and comparative analysis may further elucidate the role of specific bacterial regulatory proteins in microbial infection.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC7

The Gene Expression Response of Lung Epithelium to Infection by *Pseudomonas aeruginosa*

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In the healthy individual, host defence mechanisms usually lead to the rapid clearance of bacteria from the respiratory tract. However, in the cystic fibrosis (CF) patient several opportunistic pathogens, in particular *Pseudomonas aeruginosa* frequently colonise the lungs inducing chronic infections in the respiratory tract. In response to the infection the CF host elicits a neutrophil dominated inflammatory response which damages the airway epithelium leading to lung degeneration.

This study aims to investigate CF host gene transcriptome changes in response to infection compared to control CF cells. To identify the genes implicated in the immune and subsequent inflammatory response to infection by *P. aeruginosa w*e cultured a Cystic Fibrosis Tracheal Epithelium (CFTE29o-) cell line for co-culture with a wild-type strain of *P. aeruginosa,* PAO1.

Co-cultures were performed for a duration of 3 hours, with a multiplicity of infection (M.O.I) of bacterial cells to host cells of 50:1 (Ichikawa *et al.* 2000). The cell monolayer was harvested and messenger Ribosomal Nucleic Acid (mRNA) was isolated for comparative analysis. Host transcriptional profiles were assessed in triplicate using MWG Human 10K oligonucleotide arrays, where each sample was differentially labelled and hybridised to a microarray. Analysis of array images was performed using Imagene® and analysis of the data was carried out using Genesight® software.

Of the 9850 genes on the arrays, 2% were found to be reproducibly differentially transcribed following infection in the co-cultured sample compared to the unco-cultured control sample. The inflammatory cytokine, interleukin 8 (IL-8) was found to be differentially transcribed by 6 ± 0.15 fold following infection in our study. Standard deviation was determined, where n=3. While the overexpression of IL-8 is well documented in literature we also found that the expression of another CXC chemokine, growth-related oncogene-alpha (CXCL1) was induced following infection, with an average increase of 3 ± 0.25 fold. Additionally regulatory gene transcription was increased including, the activating transcription factor 4 (ATF4) by 3 ± 0.06 fold. Having identified novel genes of significant change we are currently validating the gene array results by quantative RT-PCR using samples isolated following co-culture.

In conclusion, these experiments demonstrate that there are significant differences in the gene transcription level in CFTE29o- following co-culture with PAO1 compared to control uninfected CFTE29o- cells and help to understand host defence mechanisms that occur following infection.

Ichikawa *et al.* (2000) *PNAS*(97) 17:9659-9664

DiMango *et al* (1995) *Clin Invest 96, 2204-2210*

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC8

The intestinal expression of a novel avian β-defensin antimicrobial peptide

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The β-defensins are a family of peptides with antimicrobial activity which provide a mechanism of host defence against infectious agents. They are expressed in vertebrate neutrophils and epithelial cells, and their structures are typified by six cysteine amino acids which form three di-sulphide bonds.

BLASTp searches of the chicken EST intestinal database identified a clone ChEST203o22 whose predicted amino acid sequence showed homology to that of the bovine β-defensin 9 antimicrobial peptide. Using the BLAST (tblastn) nucleic acid alignment programme chicken (c) β-defensin 9 was identified on chromosome 3 of the draft *Gallus gallus* genome. The gene comprises three exons and two introns.

Analyses of the encoded primary structure of the c β-defensin 9 peptide indicated a putative signal sequence suggesting that the peptide was secreted. Typical of cationic anti-microbial peptides, the peptide was composed of a high percentage of positively charged and hydrophobic amino acids and within this region six conserved cysteine residues were identified.

RT-PCR analyses using primers designed to cβ-defensin 9 and sequencing of the cDNA products, revealed the expression of two cDNA fragments (300 and 900bp) in the small intestine, liver, ovary and gizzard tissues of 5-day old chicks. In contrast only the 300 bp cDNA was detected in RNA isolated from the pancreas and white blood cells. These data suggest that cβ-defensin 9 gene expression involves spliced transcripts and is subject to tissue specific regulation.

To determine whether the expression of cβ-defensin 9 in the small intestine was constitutive or inducible in response to *Salmonella* infection, groups of five day-old chickens (n=3 or 4) were gavaged with either *S.enteriditis, S.typhimurium* SL1344 or PBS. The birds were sacrified after 4 days of infection and the small intestinal RNAs analysed for cβ-defensin expression using semi-quantitative RT-PCR. No change was detected in the expression of the 900bp cDNA fragment in the infected birds compared to controls suggesting that its expression was constitutive.

These data provide evidence for a novel cationic antimicrobial peptide which is part of the avian innate host defence system.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC9

Identification of enterohaemorrhagic *Escherichia coli* genes required for colonisation of the bovine intestine

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Enterohaemorrhagic *Escherichia coli* (EHEC) infections in humans are an important public health problem and are commonly acquired via contact with ruminant faeces. The serotypes that predominantly associated with human infection are O157:H7 and O26:H$^-$. These serotypes differ in their virulence for calves. The molecular mechanisms underlying the carriage and virulence of EHEC in the ruminant intestine are poorly understood.

All animal experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986 and were approved by the local Ethical Review Committee.

Histological analysis of tissues of experimentally inoculated conventional calves 4 days post-inoculation revealed extensive adherence of an EHEC O26:H$^-$ isolate to the colonic epithelium. In contrast, EHEC O157:H7 was scarcely detected on the epithelium at the same site in age-matched animals, indicating that O157:H7 and O26:H$^-$ EHEC may colonise the bovine host in distinct ways.

To identify *E. coli* O157:H7 and O26:H$^-$ genes that are required for intestinal colonisation of calves we have used signature-tagged transposon mutagenesis. To date, c. 600 O26:H$^-$ (strain 193) and c. 1900 O157:H7 (strain EDL933) transposon mutants have been screened. The site of transposon insertion was determined in 62 O26 mutants and 79 O157 mutants that were absent or poorly represented in the faeces and/or colonic mucosa.

Several mutants of each serotype were isolated with insertions in Locus of Enterocyte Effacement (LEE) genes encoding putative structural components of a Type III protein secretion system that is required for the formation of attaching and effacing lesions, indicating that the LEE-encoded Type III secretion system plays a key role in the colonisation of the bovine intestine by EHEC O26:H$^-$ and O157:H7.

Putative non-LEE-encoded Type III secreted proteins were also found to be required including NleD (O157), and homologues of PkgA (O26) and GogB (O26). Expression of type 1 fimbriae appears to be detrimental for persistence in vivo. Cytotoxins (ehxA, pssA and efa1) facilitate the colonisation of calves by EHEC O26:H$^-$, but were not detected in the O157 screen indicating that different EHEC serotypes may differ in their reliance on cytotoxins to colonise the intestines of calves. We have identified a putative fimbrial operon that is required for colonisation of the bovine intestine by both EHEC O26:H$^-$ and O157:H7.

Further colonisation was dependent on numerous of 'housekeeping genes' mainly coding for enzymes involved in central intermediary metabolism, in transport systems and cryptic phage or prophage related genes.

Single strain calf challenges were performed with O157 *escN*, *map* and *z2200* mutants, and with O26 *escN*, *fimE* and *pssA* mutants.

Our results will shed light on the molecular basis of EHEC serotype tissue tropism and facilitate the development of strategies to control EHEC in ruminants.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC10

An investigation into the signal sequence directing Type-III secretion mediated translocation of the Salmonella typhimurium effector protein SopE in mammalian cells.

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Type III secretion systems (TTSS) are virulence associated components of many Gram-negative bacteria that translocate bacterial proteins directly from the bacterial cytoplasm in to the host cell cytoplasm. Existing data on the translocated Yop proteins of Yersinia suggest the existence of two independent signals present in the amino terminus of the protein that direct secretion and translocation respectively. In Yersinia TTSS secretion is facilitated by the N-terminal mRNA sequence corresponding to the first 15 codons, while translocation is dependent on the region between amino acids 50 and 100. The Salmonella effector protein SopE has no consensus cleavable N-terminal sequence or any documented secretion signal. A chaperone, encoded by invB, is involved in the secretion/translocation of SopE1 by an unknown signal/mechanism. Previous studies have demonstrated that the N-terminal sequence of the protein is enough for secretion but the significance of the 5 mRNA sequence has not yet been determined. We have investigated the N-terminal region of SopE1 in S. typhimurium SL3261 confirming the importance of the amino acid sequence for secretion. Frameshift mutation analysis involving all or part of the N-terminal SopE sequence, has improved our insight on the conserved regions of the protein important for TTSS-dependent secretion. The significance of the 5 mRNA sequence was investigated in an attempt to identify novel consensus signal sequences implicated in translocation of SopE-like effector proteins into mammalian cells.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC11

Global Identification of Genes Regulated by luxS-Based Quorum Sensing in Salmonella

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Communication between bacteria involves the accumulation of extracellular molecules called autoinducers (AI), which leads to the regulation of gene expression once a critical threshold concentration is reached. A Vibrio harveyi bioassay was used to show Salmonella enterica serovar Typhimurium produced AI-2 like activity when grown in medium with glucose and this activity was shown to involve the enzyme LuxS. So far the transcription

of only a few genes in S. Typhimurium has been reported to be affected by the presence of AI-2. The aim of this study was to examine the global effect of AI-2 on the regulation of genes using S. Typhimurium SL1344 microarrays. A luxS mutant was constructed in S. Typhimurium SL1344 and using the V. harveyi bioassay was shown not to produce AI-2 like activity. RNA was isolated from cultures of SL1344 and the luxS mutant in late log phase and after labelling, hybridised to the SL1344 microarrays. Microarray data showed that the transcription of up to 9 % of the genome was altered by the deletion of luxS by more than two-fold. This included a number of genes involved in the pathogenicity of S. Typhimurium. Of great interest, a number of genes of unknown function (FUN genes) were identified that had altered transcriptional regulation in the mutant strain. In order to characterise these genes a panel of knockout mutants has been constructed and antibodies generated. These FUN genes are currently undergoing phenotypic characterisation.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC12

Inhibition of Pnc adhesion to host mucosa and identification of Host target proteins by Phages isolated by phage display analysis.

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Aims: Pnc adhesion to host mucosa is a prerequisite for carriage and disease development. Pnc adhesion is probably mediated by CW-L proteins. Filamentous bacteriophages have been genetically engineered so to incorporate the DNA template corresponding to the peptide displayed on its surface. We used random phage display library screening of Pnc CW-L for identification of their binding peptide in attempt to identify their receptors and develop inhibitors of Pnc adhesion.
Methods: Pnc serotype 3 (WU2) CW-L were purified by fetuin affinity chromatography, separated by 2D PAGE and sequenced. 20 proteins were detected. Among them, fructose-biphosphate aldolase (FBA) and elongation factor G (EFG) proteins, previously identified as lectins, were cloned and expressed in E. coli. Anti-FBA and anti-EFG antibodies were produced in mice and rabbits, respectively. The proteins and the antibodies were used for phage display library screening.
Results: 15 EFG and 30 FBA binding peptides were found. Five out 15 phages EFG binding peptides and 4 out of 5 phages expressing FBA binding peptide inhibited Pnc adhesion to cultured human HaCat and A549 epithelial cells, respectively. The inhibitory peptides were sequenced.
Conclusions: 1) Phage display allows identification of novel peptide inhibitor of Pnc adhesion to host mucosa. 2) We are currently analyzing the significance of these peptides and the existence of host homologous proteins.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC13

The effect of glucosamine sulphate supplementation on biomechanical parameters in humans

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Glucosamine Sulphate is widely used by athletes and sufferers of osteoarthritis (OA). Little is known about the actions of this supplement on the biomechanical performance of the human body. This study considers the effects of glucosamine on active spinal range of motion in healthy subjects.
A double blind placebo controlled trial was conducted on 38 subjects (aged 18-40yrs; written informed consent obtained) who were randomly assigned into two groups: group A (matched placebo tablets), group B (1500mg glucosamine sulphate per day). The following measurements were made within 30 minutes of the subjects rising from bed and later in the afternoon: lumbar flexion (Schober test), lower back and limb flexibility (sit and reach test). Cervical rotation was measured in the afternoon only, using a cervical range of motion device (CROM; Activator Instruments, USA). After the initial afternoon assessment, the subjects were given tablets and instructed how and when to take them. Measurements were repeated at weeks 2, 4 and 8. Two examiners performed independent assessments on each subject at each occasion. Analysis used paired t-tests (SPSS), data is presented as mean+/-1SD; Coefficient of variance.
5 subjects failed to complete the experiment: n=18 (group A) and 15 (group B). A diurnal increase in both lumbar spine flexion (Schober test) and sit and reach capability was found throughout. No change was found in the control (placebo) group from week 1 to week 8, however, there was a trend towards a decrease in lumbar flexion (both a.m. and p.m.; Table 1) in the glucosamine group. In both the Schober test and cervical rotation, the overall change reported for the glucosamine group was seen in a large proportion of the subjects in that group; 10/15 and 13/15 subjects, respectively.
Although the differences appear small, both measures of spinal flexibility showed change in the same direction, whereas the sit and reach test (mainly hamstring flexibility) showed no change. A decreased spinal flexibility might increase stability and reduce predisposition towards injury in this age group (i.e., athletes). However, further study is required to determine the presence and significance of these potential changes in both athletes and those with OA.

TEST	INITIAL ASSESSMENT	WEEK 8	Significance
Shobers (am)	0.149+/-0.01; 7%	0.148+/-0.01; 7%	p=0.07
Shobers (pm)	0.154+/-0.01m; 7%	0.153+/-0.01m; 7%	p=0.07
Sit and reach (am)	0.491+/-0.13m; 27%	0.486+/-0.12; 25%	p>0.1
Sit and reach (pm)	0.522+/-0.10m; 19%	0.518+/-0.11; 21%	p>0.1
Cervical rotation (pm)	135+/-15 degrees; 11%	127+/-17 degrees; 13%	p=0.01

Legend: Data for the glucosamine group only, reported as mean +/- 1SD; coefficient of variance for each of the measurements (n=15). Significance level (p value) was determined by paired t-test.

Both the Glucosamine and Placebo tablets were kindly supplied by Health Perception Ltd

Where applicable, the experiments described here conform with Physiological Society ethical requirements.