

of interference among community components; the nature of this interference can then be tested either by constructing synthetic communities in controlled conditions, or by further field experimentation. Above all, when perturbation responses are quantified using the indices suggested here, they can be used in a comparative way, to generate and test hypotheses regarding the manner in which different communities are organized.

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## Inhibition of experimental ascending urinary tract infection by an epithelial cell-surface receptor analogue

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It has been shown that the establishment of urinary tract infection by *Escherichia coli* is dependent on attachment of the bacteria to epithelial cells<sup>1–4</sup>. The attachment involves specific epithelial cell receptors, which have been characterized as glycolipids<sup>5–10</sup>. Reversible binding to cell-surface mannosides may also be important<sup>4,11–13</sup>. This suggests an approach to the treatment of infections—that of blocking bacterial attachment with cell membrane receptor analogues. Using *E. coli* mutants lacking one or other of the two binding specificities (glycolipid and mannose), we show here that glycolipid analogues can block *in vitro* adhesion and *in vivo* urinary tract infection.

Table 1 lists the properties of the *E. coli* strains used. *E. coli* HU 824 and HU 742 were selected after a two-step chemical mutagenesis of *E. coli* GR 12, isolated from a patient with acute pyelonephritis, as having either surface ligands attaching

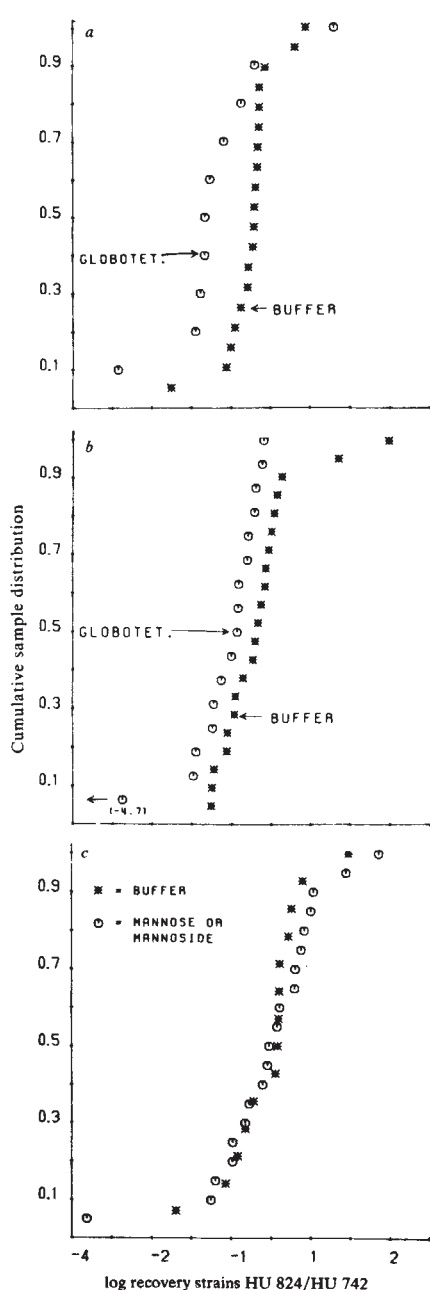
to epithelial cells through globoseries glycolipid receptors or mannose-reversible binding properties. The mutants HU 742 and HU 824 were obtained after *N*-methyl-*N*-nitro-*N*-nitrosoguanidine treatment of a lactose-negative mutant of GR 12. Further details on the construction and stability of the mutants will be reported elsewhere<sup>14</sup>. The mutants and the parent were identical by the following genotypic and phenotypic characteristics available for testing: electrophoretic mobility of 13 chromosomally determined enzymes<sup>15</sup>, three plasmid bands of the same size<sup>16</sup>, serotype<sup>17</sup>, resistance to the bactericidal effect of serum<sup>18</sup>, inability to haemolyse horse erythrocytes<sup>17</sup> and api 20 E pattern. Clearly, these tests or other techniques are insufficient to demonstrate genetic identity between the mutants. The only discernible difference between these strains was, however, the receptor specificity of their adhesins. Initially,

**Table 1** Properties of the *E. coli* strains HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup>, used for experimental ascending urinary tract infection

Property	<i>E. coli</i> strain	
	HU 824 Str <sup>R</sup>	HU 742 Nal <sup>R</sup>
	075 Knt	075 Knt
Serotype		
Antibiotic resistance		
Streptomycin	R	S
Nalidixic acid	S	R
Trimethoprim	S	S
Sulphadiazine	R	R
Tetracycline	S	S
Cephalothin	S	S
Mecillinam	S	S
Ampicillin	R	R
Nitrofurantoin	S	S
Bactericidal effect of human serum	R	R
Haemolysin production	0	0
Adhesion to uroepithelial cells (bacteria per cell)		
Human	65	0
Mouse	51	12
Receptor sugar inhibiting adhesion		
Globotetraos	9 µg ml <sup>-1</sup>	NI
α-Mn	NI	~92 µg ml <sup>-1</sup>
Agglutination of guinea pig erythrocytes		
Uncoated in PBS	–	+
in α-Mn	–	–
Coated with globotetraosylceramide		
in PBS	+	+
in α-Mn	+	–

*E. coli* HU 824 and HU 742, mutants of a wild-type pyelonephritogenic *E. coli* strain, were identical for the genotypic and phenotypic traits listed, but differed in specificity of their adhesins. R, resistant; S, sensitive; Knt, nontypable for Kantigens<sup>17</sup>. To permit separate detection out of a mixture, mutants resistant to 1 mg ml<sup>-1</sup> of streptomycin (HU 824 Str<sup>R</sup>) or to 50 µg ml<sup>-1</sup> of nalidixic acid (HU 742 Nal<sup>R</sup>) were selected by the gradient plate technique (without mutagenesis). The resistant mutants grew equally well on TSA with or without antibiotic after passage on antibiotic-free medium, retained the adhesive and haemagglutinating properties and showed no difference in *in vivo* infectivity compared with the respective parent. Adhesion to uroepithelial cells from the urinary sediment of a healthy female donor (blood group P<sub>1</sub>) and the bladder of BALB/c mice was assessed as described elsewhere<sup>1–3</sup>. Adhesion = mean no. of bacteria attached to 40 epithelial cells. For adhesion inhibition, bacteria were preincubated with decreasing concentrations of globotetraose or αMn, epithelial cells were added and adhesion testing was performed<sup>5,7</sup>. The concentration required for 50% inhibition of the adherence of 10<sup>9</sup> bacteria is shown. NI, no inhibition. Induction of binding of *E. coli* HU 824 Str<sup>R</sup> was studied by agglutination of guinea pig erythrocytes coated with globotetraosylceramide<sup>5,6</sup>. Agglutination after but not before coating, and in the presence of αMn, was credited to the receptor. The oligosaccharide globotetraose, released from globoside by ozonolysis, and purified by Folch partitioning and alkaline fragmentation, was analysed by gas-liquid chromatography and NMR (ref. 23). The NMR spectroscopy in D<sub>2</sub>O spectrum showed signals from three anomeric protons with chemical shifts, as expected for globotetraose: β-hexoseamine, β-hexose and α-hexose. The fourth anomeric signal from the reducing glucose residue was split into two, corresponding to the α and β form in the proportions 1:2.

**Fig. 1** Recovery of *E. coli* HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup> in the presence and absence of receptor sugar. The strains were grown on antibiotic-free media, collected into PBS with globotetraose (50  $\mu\text{g ml}^{-1}$ ) for expt 1 and 30  $\mu\text{g ml}^{-1}$  for expt 2, which were, respectively, four and three times the *in vitro* IC<sub>50</sub> for *E. coli* HU 824 or into PBS with  $\alpha\text{Mn}$  (5  $\text{mg ml}^{-1}$  for both experiments which was 50 times the IC<sub>50</sub> of HU 742 *in vitro* adhesion), and incubated for 60 min at 37 °C. Viable counts on the inoculum before and after incubation excluded changes in bacterial numbers due to the presence of the sugars. The inocula (per animal) for expt 1 were: PBS,  $3.9 \times 10^8 + 8.3 \times 10^7$ ; globotetraose,  $9.7 \times 10^7 + 8.3 \times 10^7$ . For experiment 2: PBS,  $3.0 \times 10^8 + 3.9 \times 10^7$ ; globotetraose,  $2.9 \times 10^8 + 3.3 \times 10^7$  of HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup> respectively. The inocula per animal for the  $\alpha\text{Mn}$  experiments were for expt 1: PBS,  $2.3 \times 10^9 + 1.4 \times 10^8$ ;  $\alpha\text{Mn}$ ,  $3.7 \times 10^9 + 1.1 \times 10^8$ . For expt 2: PBS,  $6.4 \times 10^8 + 8.3 \times 10^7$ ;  $\alpha\text{Mn}$ ,  $4.4 \times 10^8 + 1.0 \times 10^8$  of HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup> respectively. The mixed inocula with or without receptor sugar were injected into the bladder of mice which were killed 2 or 16 h later. Serial dilutions of the homogenized kidneys and bladders were cultured on TSA (BBL, Cockeysville, Maryland), TSA streptomycin (1  $\text{mg ml}^{-1}$ ) or ISA nalidixic acid (50  $\mu\text{g ml}^{-1}$ ). The relative recovery from each animal and tissue of the two strains is given as  $\log(R\text{HU } 824/R\text{HU } 742)$ . The cumulative sample distribution<sup>24</sup> of the recovery ratio is presented, starting from the animal with the lowest recovery and adding up to all animals (=100%). Both in kidneys (a) and bladders (b) a significant shift towards lower recovery of HU 824 Str<sup>R</sup> was observed after globotetraose treatment. No significant effect of  $\alpha\text{Mn}$  treatment was observed (c). The differences obtained with globotetraose were significant both at 2 and at 16 h, and the results from these animals have been pooled.



the two strains had identical antibiotic resistance patterns. To allow separate detection from a mixed inoculum, mutants resistant to 1  $\text{mg ml}^{-1}$  of streptomycin (HU 824 Str<sup>R</sup>) or to 50  $\mu\text{g ml}^{-1}$  of nalidixic acid (HU 742 Nal<sup>R</sup>) were selected (see Table 1 legend).

The difference in receptor specificity between the adhesins of *E. coli* HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup> was characterized by adhesion inhibition by soluble receptor sugars or induction of binding to previously unreactive cells by receptor coating<sup>5-7</sup> (Table 1 legend). The adhesion to human uroepithelial cells of

$10^9$  *E. coli* HU 824 Str<sup>R</sup> was reduced to 50% by 9  $\mu\text{g ml}^{-1}$  of globotetraose, the oligosaccharide obtained from globoside (a receptor glycolipid) by ozonolysis<sup>19</sup> (Table 1 legend). *E. coli* HU 742 carried mannose-binding adhesins defined by mannose-reversible agglutination of guinea pig erythrocytes<sup>21</sup>. The adhesion of *E. coli* HU 742 Nal<sup>R</sup> to human or mouse epithelial cells was inhibited by  $\alpha$ -methylmannoside ( $\alpha\text{Mn}$ ), although inhibition was too low to allow exact determination of the 50% inhibitory  $\alpha\text{Mn}$  concentration (IC<sub>50</sub>). Association with urinary slime<sup>13</sup> could not be assayed quantitatively. The agglutination by *E. coli* HU 742 of guinea pig erythrocytes was inhibited by  $\alpha\text{Mn}$  both before and after coating with globoside. *E. coli* HU 824 Str<sup>R</sup> agglutinated guinea pig erythrocytes after, but not before globoside coating. This reaction was unaffected by  $\alpha\text{Mn}$ .

Female BALB/c mice, aged 6–8 weeks, were infected by catheterization of the urethra and injection of 0.05 ml of a bacterial suspension (L.H. *et al.*, in preparation). The animals were killed at various times later, kidneys and bladders homogenized and viable counts performed. It was soon recognized that the high degree of variation among animals in the recovery of *E. coli* from kidney or bladder would tend to mask moderate differences between experimental and control groups. For this reason, each mouse was infected with a mixture of *E. coli* HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup>, separable from the mixture by their different antibiotic resistance. At the end of an experiment, kidneys and bladders of each animal were homogenized separately, and quantitative cultures of each strain prepared on agar plates containing the appropriate antimicrobial agent. The recovery, *R*, of a given strain was then determined as  $R = \text{no. of bacteria in the organ cultured}/\text{no. of bacteria in the inoculum}$ .

To evaluate the *in vivo* effectiveness of a given treatment (for example, the addition of globotetraose or  $\alpha\text{Mn}$  to the inoculum), the two *E. coli* strains were chosen such that one was susceptible and the other resistant to the *in vitro* effects of the treatment under test. The *in vivo* effectiveness of the treatment was then evaluated by comparing the recoveries of the two strains as the recovery ratio,  $R$  (susceptible)/ $R$  (resistant). Consequently, if the recovery ratio in the experimental group was significantly lower than in the control group, it could be concluded that the treatment had been effective *in vivo*. In this manner, the randomizing effects of individual differences among animals were overcome by having one *E. coli* strain serve as an intrinsic control in each animal.

Inocula consisting of *E. coli* HU 824 Str<sup>R</sup> and HU 742 Nal<sup>R</sup> were prepared as detailed in Fig. 1 legend. After incubation with globotetraose or  $\alpha\text{Mn}$ , respectively, the *in vitro* attachment to mouse epithelial cells and agglutination of human or guinea pig erythrocytes was abolished. The inocula in phosphate-buffered (saline PBS) retained these properties. After intravesicular injection of the inocula, the animals were killed and viable counts of the homogenized kidneys and bladders on trypticase soy agar (TSA), TSA-streptomycin or TSA-nalidixic acid were performed. The result of globotetraose treatment is shown in Fig. 1a for kidney cultures and in 1b for bladder cultures, while Fig. 1c shows the result of  $\alpha\text{Mn}$  treatment. The recovery of *E. coli* HU 824 Str<sup>R</sup> was significantly reduced, both in kidneys and bladders after globotetraose treatment, as seen by the shift of the cumulative sample distribution curve towards the left ( $P = 0.019$ , Mann-Whitney *U*-test). After  $\alpha\text{Mn}$  treatment the opposite effect, a decrease of HU 742 Nal<sup>R</sup> recovery with a shift of the cumulative sample distribution to the right, might have been expected. No significant difference from the PBS control was observed.

We propose the following interpretation of the results. Capacity to attach to the mucosal lining of the mouse urinary tract was an important survival mechanism for *E. coli* HU 824, as seen by its efficient adherence to mouse bladder cells *in vitro* and the decrease of *in vivo* infectivity after pretreatment with globotetraose. *E. coli* HU 742 attached in low numbers *in vitro* to the mouse bladder cells, but might bind to urinary slime. An inhibitory effect of  $\alpha\text{Mn}$  *in vivo*, as reported in other systems<sup>20</sup>,

may be masked by a dual effect of  $\alpha$ Mn. Blocking of binding to mucus may facilitate the ascent of bacteria to the kidney, as well as increase the elimination of bacteria from the urinary tract. The inhibition of experimental infection by globotetraose, on the other hand, illustrates a new approach to prophylaxis and treatment of infections occurring via mucous membranes. Compounds mimicking the host receptors may competitively bind to bacterial surface ligands<sup>20,22</sup>. This might decrease the number of bacteria attaching to the mucosa sufficiently to alter the delicate balance of host-parasite interaction in favour of the host.

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## A stem cell for stem cells in murine haematopoiesis

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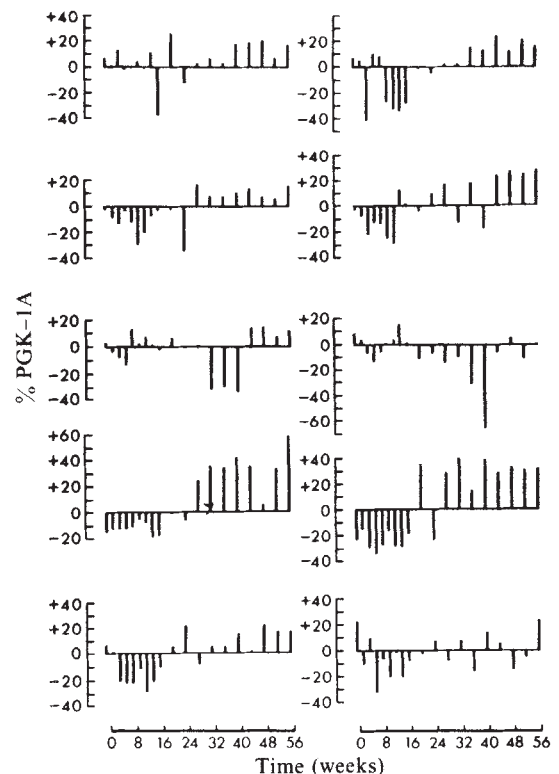
**Mature erythrocytes and granulocytes have limited lifespans, do not replicate and must therefore be replenished constantly. They are derived from pluripotent stem cells (PSCs) which are capable of self-renewal<sup>1</sup>. The numbers and properties of PSCs can be inferred in part from studies of their progeny. Such studies have depended largely on highly artificial experimental systems, involving such procedures as X-ray irradiation, bone marrow transplantation and parabiosis<sup>2</sup>. We now describe a method for studying the behaviour of haematopoietic cell populations in normal mice, and show that erythropoiesis is maintained by the products of a very small number of clones which, as predicted by Kay<sup>3</sup>, arise and decline in succession. These results suggest that spleen colony-forming cells (CFC), usually regarded as stem cells, are themselves members of substantial clones which differentiate in sequence.**

In female mammals, one of the two X chromosomes becomes irreversibly inactivated early in embryogenesis; subsequently any somatic cell and its descendants fail to express genes carried

on the inactivated chromosome<sup>4,5</sup>. X-linked genetic variants can therefore provide markers for cell population studies, and have been used to estimate embryonic founder cell numbers for the haematopoietic system<sup>6,7</sup>. Recently, the discovery of an electrophoretic variant of the X chromosome-encoded enzyme phosphoglycerate kinase (PGK-1)<sup>8</sup>, and the development of improved methods for measuring PGK activity in electrophoresis strips<sup>9</sup>, have provided a convenient marker system in the mouse.

Female mice heterozygous at the PGK-1 locus were bled at 14-day intervals for 16 weeks and then at 28-day intervals for a further 40 weeks. The enzyme phenotypes were determined electrophoretically from haemolysates using a modification of the technique of Bücher *et al.*<sup>9</sup>.

The percentages of PGK-1A alloenzyme in serial blood samples from 10 normal heterozygous mice are shown in Fig. 1. Large, and frequently sudden, variations in the relative amounts of A and B alloenzymes are evident in every individual,



**Fig. 1** Percentages of PGK activity attributable to the A alloenzyme in serial blood samples from 10 PGK-1A/B heterozygous female mice. The data for each mouse are plotted as the deviation of individual samples from the overall percentage of A alloenzyme in the mouse; this was estimated as the mean of the readings obtained on day 0 and at 56-day intervals thereafter (see text). The percentages of A and B alloenzymes were determined electrophoretically from haemolysates as described by Bücher *et al.*<sup>9</sup> with the following modification. After electrophoresis, the Cellogel electrophoresis strips were applied to a polyethylene imine (PEI) TLC sheet on which had been spread a solution of the enzymes, substrates and co-factors necessary for the visualization of PGK activity<sup>9</sup>, with added <sup>14</sup>C-labelled D-glucose. The radioactive final products of the linked enzyme reactions (glucose-6-phosphate and 6-phosphogluconolactone) were adsorbed onto the PEI sheet<sup>14</sup>, which was then autoradiographed on Kodak XS X-ray film. The autoradiographs were scanned with a double-beam recording densitometer and the area under each peak integrated. The figures obtained were corrected to compensate for the log-linear response of the X-ray film to exposure, as determined by calibration with a series of known alloenzyme mixtures, and the proportions of the A and B alloenzymes were calculated. In most animals the overall A:B ratio was ~7:3, a value consistent with the expected linkage of *xce* alleles (controlling the probability of a given X chromosome escaping inactivation) to the PGK-1A and 1B alleles<sup>15</sup>. The mice used were from the eighth generation back-cross of PGK-1A onto the CBA/Ca (PGK-1B) strain.